Muscle Fatigue and Motor Output Variability with Acute Stress in Healthy Young Adults and Veterans with Posttraumatic Stress Disorder

Manda Linea Keller
Marquette University

MUSCLE FATIGUE AND MOTOR OUTPUT VARIABILITY WITH ACUTE STRESS IN HEALTHY YOUNG ADULTS AND VETERANS WITH POSTTRAMATIC STRESS DISORDER

by

Manda L Keller, DPT

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ABSTRACT
MUSCLE FATIGUE AND MOTOR OUTPUT VARIABILITY WITH ACUTE STRESS IN HEALTHY YOUNG ADULTS AND VETERANS WITH POSTTRAMATIC STRESS DISORDER

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Acute stress can alter motor performance differently for men and women. The first aim of this dissertation addresses possible causes for the sex difference in the motor response of a low-intensity fatiguing contraction of the elbow flexor muscles to an acute stressor (difficult mental math) in young, healthy adults. Muscle fatigue increased for men and women when exposed to the stressor, but impairment was more prominent for the women. This work showed that fatigue in the central nervous system, specifically in cortical motor and premotor areas, as well as relaxation rates of the muscle (quantified with cortical stimulation) were not responsible for the stress-induced motor fatigue. The greater fatigue with stress was associated with the strength of the individual such that weaker individuals (mainly women) fatigued more quickly when exposed to the stressor than stronger individuals (mainly men). Thus, the mechanism of stress-induced fatigability in weaker subjects may be due to differences in blood perfusion to the muscle. Furthermore, women were less steady than men for very low-intensity contractions in the presence and absence of stress. Steadiness was impaired at failure of the fatiguing contraction but persisted more for women than men, up to 20 minutes recovery.

A second aim was to determine muscle fatigability and steadiness in veterans with posttraumatic stress disorder (PTSD) in the presence and absence of the cognitive stressor. Male veterans with PTSD and male civilian controls performed a low-intensity contraction till task failure with the handgrip muscles. Veterans with PTSD fatigued more quickly and were less steady than the control subjects. When exposed to a cognitive stressor, neither the veterans with PTSD nor control subjects had greater fatigability or reduced steadiness. Veterans with PTSD however, were more fatigable compared with the control subjects in both stressful and non-stressful conditions, suggesting that the chronic stress condition (vs. acute stress) has a greater influence on motor performance for the hand muscles. Understanding how motor control is altered in the presence of acute stress and in clinical populations can lead to more tailored treatment interventions for optimized rehabilitation programs for specific motor impairments and for clinical populations.

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LIST OF ABBREVIATIONS

ACTH = Adrenocorticotrophin Releasing Hormone

ADP = Adenosine Diphosphate

ATP = Adenosine Triphosphate

BDI = Beck Depression Inventory

BMI = Body Mass Index

Ca^{2+} = Calcium

CV = Coefficient of Variation

CMEP = Cervicomedullary Motor Evoked Potential

CRH = Corticotrophin Releasing Hormone

DBP = Diastolic Blood Pressure

EMG = Electromyography

eRT = Estimated Resting Twitch

ESTIM = Brachial plexus stimulation

H^{+} = Hydrogen ion

HPA = Hypothalamic Pituitary Adrenal

LC = Locus Coeruleus

MA = Mental Attentiveness

MAP = Mean Arterial Pressure

METS = Metabolic Equivalents

MM = Mental Math

MVC = Maximal Voluntary Contraction

M wave = Compound Muscle Action Potential

MEP = Motor Evoked Potential

NE = Norepinephrine

NO = Nitric Oxide

PA = Physical Activity Questionnaire

PCL-C = Posttraumatic Stress Disorder Checklist- Civilian

P_i = Inorganic Phosphate

PFC = Prefrontal Cortex

POMC = Pro-opiomelanocortin

PRR = Peak Relaxation Rates

PTSD = Posttraumatic Stress Disorder

RMS = Root Mean Squared

RPE = Rating of Perceived Exertion

RPP = Rate Pressure Product

SBP = Systolic Blood Pressure

SD = Standard Deviation

SEM = Standard Error Measurement

SIT = Superimposed Twitch

SSRI = Selective Serotonin Reuptake Inhibitor

SNRI = Selective Norepinephrine Reuptake Inhibitor

STAI = State Trait Anxiety Inventory

TMS = Transcranial Magnetic Stimulation

VAMC = Veteran Affairs Medical Center

VAS = Visual Analogue Scale

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Chapter I

Introduction

Motor performance during sustained contractions is important for functional tasks and neuromuscular rehabilitation. Low-intensity fatiguing contractions for example, are the foundation for daily tasks such as postural contractions and stabilizing the upper limb while performing fine motor tasks. Sustaining low-intensity contractions can lead to muscle fatigue and reduced steadiness during target matching tasks. Muscle fatigue is an exercise-induced reduction in muscle force or power caused by impairments within the neuromuscular system, which is reversible with rest (Fitts, 1994; Enoka & Duchateau, 2008). It can be quantified as a loss of maximal strength or an inability to maintain the required force during a sustained contraction and is specific to the individual, the requirements of the task and conditions in which the task is performed. One example is that women are less fatigable than men, although the sex difference can vary with the task performed (Hunter, 2009). In general, the sex differences are not fully understood but are thought to be primarily due to muscular (peripheral) mechanisms, although the role of supraspinal mechanisms is not known. The first study investigates the role of supraspinal fatigue to the sex differences in neuromuscular fatigue for low-intensity contractions.

Motor output variability will increase with fatigue and can vary between men and women and under different conditions. While maintaining a steady contraction for example, the force exhibited by the muscle fluctuates about a target force and this represents the variability in motor output. Fluctuations in force are quantified by normalizing the standard deviation of the force to

the mean of the force and represented as the coefficient of variation (CV) (Galganski et al., 1993). Additionally, there are sex differences in motor output variability during low-intensity isometric contractions. Women are less steady and this is indicated by an increase in the coefficient of variation of the force signal (Brown et al., 2010). One factor that may alter neuromuscular fatigue and motor output variability, particularly in women, is exposure to an acute stressor (increased arousal) (Noteboom et al., 2001a; Christou et al., 2004; Yoon et al., 2009). The mechanisms contributing to the sex differences in muscle fatigue and motor output variability when exposed to an acute stressor are not well understood and will be examined in the second and third studies of this dissertation.

Additionally, exposure to an acute traumatic stress may result in adaptations within the sympathetic nervous system which may be important for motor control. Posttraumatic Stress Disorder (PTSD) is a chronic stress disorder that can occur after exposure to an acute traumatic event. The adaptations that occur with PTSD are known to affect the sympathetic and neuroendocrine systems (Pervanidou & Chrousos, 2011) and are likely to affect the neuromuscular system; however, the influence of PTSD on motor performance has not been studied. Hence, the fourth study will address how having PTSD, specifically in combat veterans, may affect muscle fatigability and motor output variability in the presence and absence of an acute stressor for low-intensity tasks with the hand muscles.

Section I. Muscle Fatigue and Motor Output Variability

Muscle Fatigue

The magnitude and mechanisms of muscle fatigue are dependent on the individual and specifics of the task being performed. Most often, muscle fatigue is quantified by the reduction in maximal strength after a submaximal or maximal fatiguing task but can also be quantified by

an individual's ability to maintain a contraction force (Enoka & Duchateau, 2008). There are several sites along the neuromuscular system that may contribute to muscle fatigue. These include 1) suboptimal activation of the motor cortex (Gandevia et al., 1996; Todd et al., 2003), 2) decreased neural drive to the motoneuron and the activation of the motoneuron (Merton, 1954; Herbert & Gandevia, 1999), 3) impairment of the propagation of the action potential at the neuromuscular junction (Fuglevand et al., 1993), 4) compromised blood perfusion to the muscle (Thompson et al., 2007), 5) disruptions in excitation-contraction coupling (Fitts, 1994), 6) differences in muscle metabolism and contractile properties (Allen et al., 2008b) and 7) altered cross-bridge mechanics (adenosine triphosphate (ATP) turnover) during a contraction (Fitts, 2008). When the demands of the task are different, the site that is the most stressed will also differ (Enoka & Duchateau, 2008). Therefore, the mechanisms that limit the force production and the magnitude of the fatigue can vary with the demands of the task. Figure 1.1 denotes the steps involved in voluntary force production and possible mechanisms of muscle fatigue.

Central Contributions to Muscle Fatigue

Central fatigue contributes to neuromuscular fatigue during maximal and submaximal fatiguing contractions (Gandevia, 2001). It is defined by impairments in the neuromuscular system that occur proximal to the neuromuscular junction and is a progressive exercise-induced reduction in voluntary activation of the muscle (Gandevia et al., 1995). This can be measured by imposing supramaximal stimulation to the motor nerve of the muscle during a maximal voluntary contraction (MVC). Extra force evoked by the superimposed stimulus to the axons indicates that either the motor units were not all recruited voluntarily or discharge rates were not high enough to produce full fusion of force (Merton, 1954; Belanger & McComas, 1981; Herbert & Gandevia, 1999).

Supraspinal fatigue is a component of central fatigue and is quantified by transcranial magnetic stimulation (TMS) also known as motor cortical stimulation. It is defined as suboptimal output from the motor cortex or upstream of the motor cortex (Gandevia, 2001). Supraspinal fatigue is measured as an exercise-related decline in voluntary activation. Voluntary activation however, measured with motor cortical stimulation, reveals something different than when measured with motor nerve stimulation. If stimulation at the motor cortex evokes a superimposed twitch (SIT) during a MVC, then motor cortical output was not maximal and not sufficient to drive the motoneurons maximally. In turn, motoneuron firing was not maximal or sufficient to drive the muscle maximally (Taylor et al., 2006). Under some conditions, central fatigue is responsible for 20-25% of the loss of force with contributions from supraspinal sources after maximal and submaximal fatiguing contractions (Taylor et al., 2006). Additionally, supraspinal fatigue appears to have larger contributions to submaximal fatiguing contractions compared with maximal fatiguing contractions (Taylor & Gandevia, 2008). In other words, the reduction in voluntary activation measured by cortical stimulation may be greater for low-intensity fatiguing contractions compared with high-intensity fatiguing contractions.

Adjustments in the electromyography (EMG) during fatigue can exhibit valuable information regarding motor unit activity (motor unit recruitment and discharge rates of motor units) and factors that modulate the characteristics of the motor unit action potentials in the muscle (Dideriksen et al., 2011). During sustained contractions, motoneuron activity (located within the dorsal horn of the spinal cord) will eventually slow and some motoneurons may stop firing. This will lead to changes in the EMG signal (Peters & Fuglevand, 1999; Sacco et al., 2000). Motoneuron excitability can be altered with fatigue and is measured by the cervicomedullary motor evoked potential (CMEP) which is the EMG response to the

cervicomedullary stimulation. The CMEP is the short-latency excitatory response to stimulation at the corticospinal tracts elicited at the level of the cervicomedullary junction (Ugawa et al., 1991). During fatigue, there are changes in the input to the motoneuron, such as greater inhibition from the group III and IV afferents, enhanced presynaptic inhibition to the Ia afferents, reduced feedback from the muscle spindle and reduced descending drive (Taylor & Gandevia, 2008). Recurrent inhibition may also play a role in fatigue and may be muscle dependent, but less is known about this mechanism (Katz & Pierrot-Deseilligny, 1999). This altered input at the motoneuron can be represented by a decrease in the CMEP after a sustained fatiguing contraction and may contribute to greater central fatigue (McNeil et al., 2009; McNeil et al., 2011).

Changes in excitability of the corticomotor tract are suggested during sustained fatiguing contractions. Corticospinal excitability can be quantified by the motor evoked potential (MEP). The MEP is the short-latency excitatory response to the stimulation at the motor cortex, which has been shown to increase with fatigue (Taylor & Gandevia, 2001) and indicates excitation of the motor cortex and motoneuronal activity (Taylor et al., 1996). The increase in MEP with fatigue likely indicates an increase in descending drive to compensate for the fatigue-induced decrease in spinal excitability (Gandevia, 2001).

Additionally, the silent period duration in the EMG increases during a submaximal fatiguing contraction, which indicates greater inhibition within the corticospinal pathway (Taylor et al., 1996). The silent period immediately follows the MEP and is a silencing of the EMG activity. The silent period indicates complete inhibition of motor activity which can last up to more than ~200 ms. The earlier part is due to both cortical and spinal mechanisms, but the latter (>100 ms) is considered to be inhibition from the motor cortex (Taylor & Gandevia, 2001). However, McNeil et al. (2009, 2011), recently found that the first 100 ms of the silent period was

due to inhibition within the spinal cord. In other words, the silent period may be more representative of that which is occurring at the spinal cord level vs. the motor cortex. The balance of corticospinal excitability and inhibition is important for motor control and the changes measured by TMS indicate important alterations within the corticomotor system during sustained contractions as fatigue progresses. Corticospinal excitability (MEP) likely increases during a fatiguing contraction because of the increased central drive needed to compensate for the reduction in motoneuron excitability.

Changes in the EMG response and the increase in the superimposed twitch force (or decrease in calculated voluntary activation) during sustained contractions can be dissociated. That is, the EMG response to TMS (MEP) recovers rapidly after a fatiguing contraction, but supraspinal fatigue (voluntary activation) recovers more slowly (Gandevia et al., 1996; Taylor et al., 1996). After a fatiguing contraction, the slow recovery in supraspinal fatigue may be due to activation of the small muscle afferents (group III and IV) which may inhibit cortical drive and slow the recovery of supraspinal fatigue (Butler et al., 2003). In contrast, the corticospinal and motoneuron excitability appear to recover quickly (Taylor & Gandevia, 2008) as indicated by the recovery of the MEP.

Peripheral Contributions to Neuromuscular Fatigue

Peripheral (muscular) fatigue is typically defined by fatigue that occurs at or distal to the neuromuscular junction (Fitts, 2011). Neuromuscular transmission failure does not appear to be impaired during or after fatigue (Bigland-Ritchie et al., 1982; Bigland-Ritchie & Woods, 1984) and therefore the sites of muscle fatigue occur distal to the neuromuscular junction (Fitts, 1994). Mechanisms contributing to peripheral muscle fatigue will depend on the task performed, such as a high- vs. low-intensity exercise and short- vs. long-duration exercise (Fitts, 1994). For

example, cellular mechanisms may have greater contributions to muscle fatigue for high-intensity exercise compared with lower-intensity sustained contractions where blood perfusion, or lack thereof, may have a stronger contribution to muscle fatigue.

When studying the mechanisms of muscle fatigue, the fiber type proportion should be considered. Skeletal muscle contains Type I and Type II (a, b and x) fibers which have different fatigue characteristics. Type I fibers are slow-oxidative, fatigue-resistant fibers with low-thresholds that are recruited first during a low-intensity contraction. Type II fibers are fast-twitch, fast-fatigable fibers with high-thresholds that are recruited later during low-intensity sustained contractions as well as immediately during high-intensity contractions (Henneman, 1985). Compared with Type II fibers, Type I fibers in animal muscle have a greater mitochondrial enzyme content, low sarcoplasmic reticulum and ATPase activity, slower speed of contraction and slower rate of energy utilization (Fitts, 1994; Stienen et al., 1996; Hamada et al., 2003) and characteristically fatigue at a different rate (Gordon et al., 1990; Hamada et al., 2003).

Cellular aspects of muscle fatigue can occur from impairment in the mechanics of the cross-bridge cycle, excitation-contraction coupling and/or cell metabolic pathways (Figure 1.1) (Fitts, 2008). When performing a muscle contraction, adenosine triphosphate (ATP) hydrolysis occurs to produce energy for cross-bridge cycling, resulting in increases in adenosine diphosphate (ADP), inorganic phosphate (P_i) and hydrogen (H^+) (Fitts, 2008). These levels increase and levels of muscle high-energy phosphates, ATP and phosphocreatine decrease as fatigue develops during high-intensity exercise (Fitts, 1994; Allen et al., 1995). The initial decline in force with stimulation of muscle fibers occurs with no change in calcium (Ca^{2+}) and is thought to be mediated by the combined effects of an increase in P_i and H^+ (Allen et al., 1995). The increase in concentrations of P_i and H^+ potentially inhibit or reverse the cross-bridge

transition from the low- to high-force state and/or by reducing the force per cross bridge (Fitts, 2008).

Impairment may also occur with excitation-contraction coupling during high-intensity exercise. The major components involved are the surface membrane, the t-tubules and the sarcoplasmic reticulum membrane, including Ca^{2+} release channels (ryanodine receptor) and the Ca^{2+} pump proteins (Fitts, 1994; Allen et al., 2008a). The twitch force (P_t), determined by electrically stimulating the muscle fiber (or resting twitch if stimulating the muscle) is a useful technique in identifying cellular mechanisms of fatigue. In general, the P_t is depressed after fatiguing contractile activity. However, since P_t is influenced by muscle temperature, rate of force development and the duration of the intracellular Ca^{2+} transient, it does not always reflect the degree of fatigue-induced decline in either the number of crossbridges that can be activated or the force per bridge (Fitts, 2011). Other features of a twitch post fatigue are prolonged contraction (CT) and ½ RT and reductions in the peak rate of tension development and decline. Relaxation of the muscle fiber appears to slow in direct proportion to the degree of fatigue. Therefore, the changes in twitch with fatigue reflect the intracellular Ca^{2+} transient which has reduced amplitude, a slower onset and rate of decline and prolonged duration in fatigue muscle fibers (Fitts, 2011).

In humans, the resting twitch amplitude can be measured either directly by supramaximal stimulation of the muscle at rest and may provide insight into the extent to which muscle fibers are recruited by volition (central nervous system drive). The twitch can be estimated using TMS during a series of brief contractions above 50% of MVC force. Estimating the resting twitch is reliable in both fresh and fatigued muscle and may provide information regarding the recruitment of motor units by volition (Todd et al., 2003).

The motor unit is the motoneuron and all of the muscle fibers that the motoneuron innervates (Fulton, 1931). Consistent with whole muscle experiments, single motor units show prolonged contraction and relaxation times, reduced rates of tension development and decline, and a reduced shortening speed following fatigue (Fitts, 1994). Consequently, Type I motor units will demonstrate slower rates of fatigue compared to Type II motor units. Therefore, a muscle with a greater proportion of Type I motor units will demonstrate reduced fatigability compared with a muscle that contains a greater proportion of Type II motor units.

Adequate blood perfusion is important in providing the muscle with oxygen and nutrients that will slow processes of fatigue. Lack of blood perfusion to the muscle can be a source of fatigue for tasks such as sustained contractions (Bigland-Ritchie et al., 1995). This is because as the contraction force is maintained, mechanical compression of the arterioles that supply the exercising muscle will occlude blood flow and lead to increased fatigability. Therefore, blood perfusion is less and fatigability greater for stronger muscles and higher contraction forces (Hunter & Enoka, 2001).

In summary, the mechanisms of muscle fatigue are complex and depend on the type of exercise, the individual and the fiber type composition of the muscle. Several factors both central and peripheral as described in this section can contribute to fatigability of the muscle. The next section will discuss how the sex of the individual may influence factors of muscle fatigue.

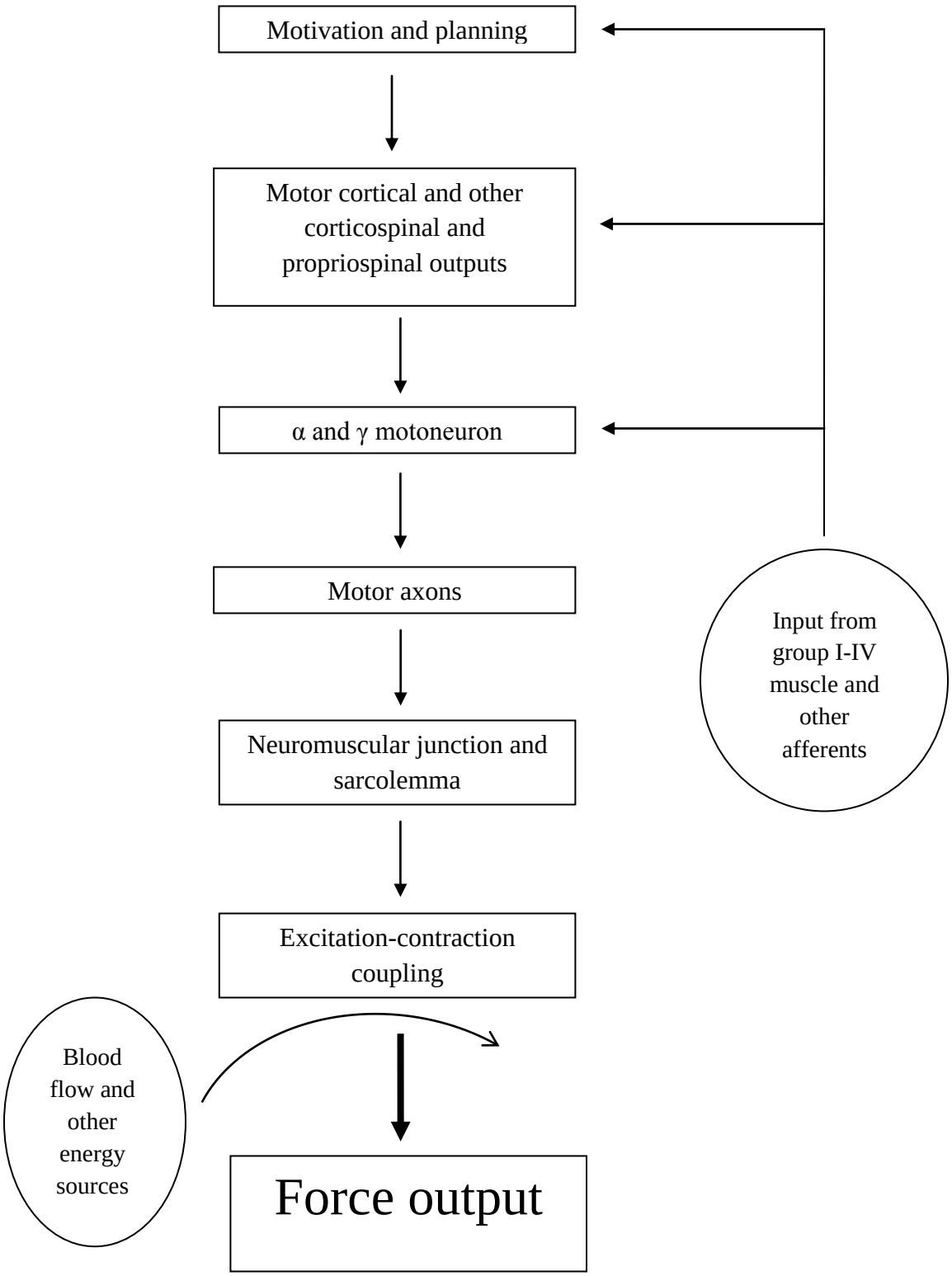

Figure 1.1. Steps involved in voluntary force production and potential mechanisms of muscle fatigue. Modified from Gandevia *Physiol Rev*. 2001.

Sex Differences in Muscle Fatigue

Sex differences in muscle fatigue have been widely established and varying the task requirements has provided insight into mechanisms that contribute to the differences in fatigability in men and women (Hunter, 2009). For example, women are able to sustain a longer time to failure than men for a relative low-intensity contraction (Petrofsky et al., 1975; Petrofsky & Phillips, 1980; Kahn & Monod, 1984; Hunter et al., 2004a; Clark et al., 2005; Hunter et al., 2006b), but not at a higher intensity contraction (80% of maximal voluntary contraction, MVC) (Yoon et al., 2007). Likewise, women experience less of a reduction in maximal force after sustained or intermittent maximal fatiguing contractions (Russ & Kent-Braun, 2003; Hunter et al., 2006a). The mechanisms contributing to these sex differences are not completely elucidated and appear to depend on the type and intensity of contraction as well as the muscle being tested.

Low-intensity contractions are relevant to daily functional activities and can be used in a laboratory setting to understand the mechanisms of muscle fatigue under different conditions. A recent review on the sex differences in muscle fatigue determined that for sustained isometric contractions, women were 23% less fatigable and for intermittent isometric contractions women were 33% less fatigable than men (Hunter, 2009). The magnitude of sex differences for time to task failure during sustained isometric contractions is inversely related to the contraction intensity. Therefore, it is expected that the greatest sex differences in time to failure would be at low-intensity contractions. Consistent with this hypothesis, the time to failure for a contraction sustained at 20% of MVC exhibited a 38% sex difference in time to failure, where as a contraction maintained at 80% of MVC force demonstrated a 3% difference in time to failure for men and women (Yoon et al., 2007). Consequently, the sex difference in time to failure for a submaximal fatiguing contraction is diminished for high-intensity isometric contractions. Figure

1.2 represents a model to show the potential mechanisms that may contribute to the sex differences in muscle fatigue for low-intensity isometric contractions (Hunter, 2009).

The inverse relationship between the sex difference in muscle fatigue and contraction intensity is related to the absolute strength in men and women when exerting the same relative contraction intensity (Hunter & Enoka, 2001). In most cases, men are stronger than women and generate a greater absolute force during the same relative strength contraction. The larger absolute force will create a greater mechanical compression to the vasculature and will decrease blood perfusion to the muscle more so for the men (Barnes, 1980). In order to determine if the difference in blood perfusion contributes to sex differences in neuromuscular fatigue, Hunter et al. (2004b) matched 10 men and 10 women for strength to perform a fatiguing contraction at 20% of MVC with the elbow flexor muscles. In contrast to when men were stronger than women, there was no sex difference in time to failure when matched for strength (13.6 ± 5.1 minutes for men and 14.4 ± 6.5 minutes for women). Rating of perceived exertion, heart rate and torque fluctuations were similar for men and women, demonstrating similarities in descending drive (Hunter et al., 2004a). The pressor response was also similar; the pressor response is a reflex-mediated increase in mean arterial pressure due to metabolite accumulation that attempts to rectify the mismatch between perfusion and metabolism during an isometric contraction (Mitchell et al., 1983; Rowell & O'Leary, 1990). This suggests that the intramuscular pressure during the contraction was similar. The similarities in physiological variables for men and women when matched for strength are in contrast to when men are stronger than women as the heart rate, torque fluctuations and pressor response are all greater or increase at a faster rate for men compared with women (Hunter & Enoka, 2001). Similar to when matched for strength, occlusion of the muscle during a fatiguing contraction diminishes the sex difference in muscle

fatigue (Clark et al., 2005). Greater blood occlusion in stronger muscles leads to more accumulation of metabolites, impairment of oxygen delivery to the muscle and a more rapid rate of muscle fatigue (Hicks et al., 2001).

Sex Differences in Muscle Metabolism and Contractile Properties

There are sex differences in fiber type proportion and muscle metabolism that may contribute to the sex differences in muscle fatigue during low-intensity contractions. Consistent with a higher glycolytic metabolism, men have greater muscle cross-sectional area and a greater Type II/Type I ratio for the tibialis anterior (Jaworowski et al., 2002), vastus lateralis (Green et al., 1984) and the gastrocnemius muscles (Coggan et al., 1992), demonstrating that the sex difference in muscle metabolism may not be muscle specific. Muscle in women is on average slower than muscle in men (see Figure 1.2) (Hunter et al., 2006a; Wust et al., 2008).

The differences in contractile properties and energy utilization may play a significant role in the sex difference during fatiguing contractions. Contractile properties can be measured either by electrical stimulation of the resting muscle or cortical stimulation during voluntary contractions in humans. Using TMS, peak relaxation rates of the whole muscle can be quantified (Todd et al., 2004) and likely reflects the proportional area of fiber types in the muscles. For example, peak rates of relaxation in the elbow flexor muscles are faster in men ($-13.5 \pm 2.2\ s^{-1}$) compared with women ($-9.3 \pm 1.8\ s^{-1}$) during maximal strength contractions and the peak relaxation rates slowed more for the men (53% decline) than the women (22% decline) for a maximal fatiguing contraction (Hunter et al., 2006a). Consistent with these findings, peak relaxation rates, measured with electrical stimulation of the motor nerve, are also found to be slower in women compared with men in the knee extensor muscles (Wust et al., 2008). Wust et al., (2008) used an electrical stimulation protocol to fatigue the muscle and found that women

were more fatigue resistant than the men and the magnitude of fatigue was associated with the peak relaxation rates of the muscle, suggesting those that had faster peak relaxation rates had a greater decline in force. This indicates that the sex difference in peak relaxation rates may be contributing to the sex difference in muscle fatigue for isometric contractions.

Consistent with a greater muscle cross-sectional area, the estimated resting twitch amplitude is greater in young men than women in the elbow flexor muscles and also has greater reductions in men (59%) compared with women (27%) during maximal isometric fatiguing contractions (Hunter et al., 2006a). Therefore, for fatiguing contractions at maximal strength, men appear to have greater declines in the peak relaxation rates and twitch amplitudes compared with women. The changes in peak relaxation rates and the resting twitch amplitude in men and women after low-intensity fatiguing contractions are unknown and will be quantified in the first study of this dissertation.

Sex Differences in Central Fatigue

Fatigue that occurs proximal to the neuromuscular junction is known as central fatigue and can be different in men and women depending on the muscle group performing the task. For example, men had greater reductions in strength compared with women for an intermittent maximal isometric fatiguing contraction of the dorsiflexor muscles that was partially due to greater central impairment in men compared with women (Russ & Kent-Braun, 2003). Similarly, a sustained maximal contraction induced greater reductions in strength for men (24%) compared with women (16%) and larger reductions in voluntary activation in men (22% vs. 9%, respectively) for the knee extensor muscles (Martin & Rattey, 2007). The reason for the greater central impairment in men is unknown, but may be due to greater negative feedback to the

motoneuron or other descending inputs (within the motor cortex) from firing of group III and IV muscle afferents (see Figure 1.2) (Gandevia, 2001; Martin et al., 2008).

In contrast, there was no sex difference demonstrated in supraspinal fatigue with the elbow flexor muscles during maximal sustained contractions (Hunter et al., 2006a). Men demonstrated greater reductions in strength which was associated with greater reductions in the peak relaxation rates and estimated resting twitch, indicating that the greater fatigue in men was due to peripheral factors. Furthermore, MEP and silent period increased similarly for men and women during the fatiguing contraction indicating similar increases in corticospinal excitability and inhibition. Similarly, there was no sex difference in the reduction of voluntary activation for 20 and 80% of MVC submaximal fatiguing contractions of the elbow flexor muscles using motor nerve stimulation (Yoon et al., 2007).

Collectively, the sex differences in fatigability appear to be task dependent and rely on both central and peripheral mechanisms. This includes differences in supraspinal fatigue, blood perfusion to the muscle, differences in glycolytic metabolism and proportion of fast fatigable fiber types. More importantly, these studies suggest that more than just one mechanism is involved in the sex differences in muscle fatigue for low-intensity fatiguing contractions (see Figure 1.2) (Hunter, 2009). In the current literature, it is understood that there are no sex differences in supraspinal fatigue for maximal fatiguing contractions with the elbow flexor muscles, but the contribution of supraspinal fatigue to the sex differences in neuromuscular fatigability for a low-intensity contraction, is unknown. Therefore, one of the aims for the first study is to understand the supraspinal contributions to the sex differences in muscle fatigability for a sustained low-intensity contraction with the elbow flexor muscles.

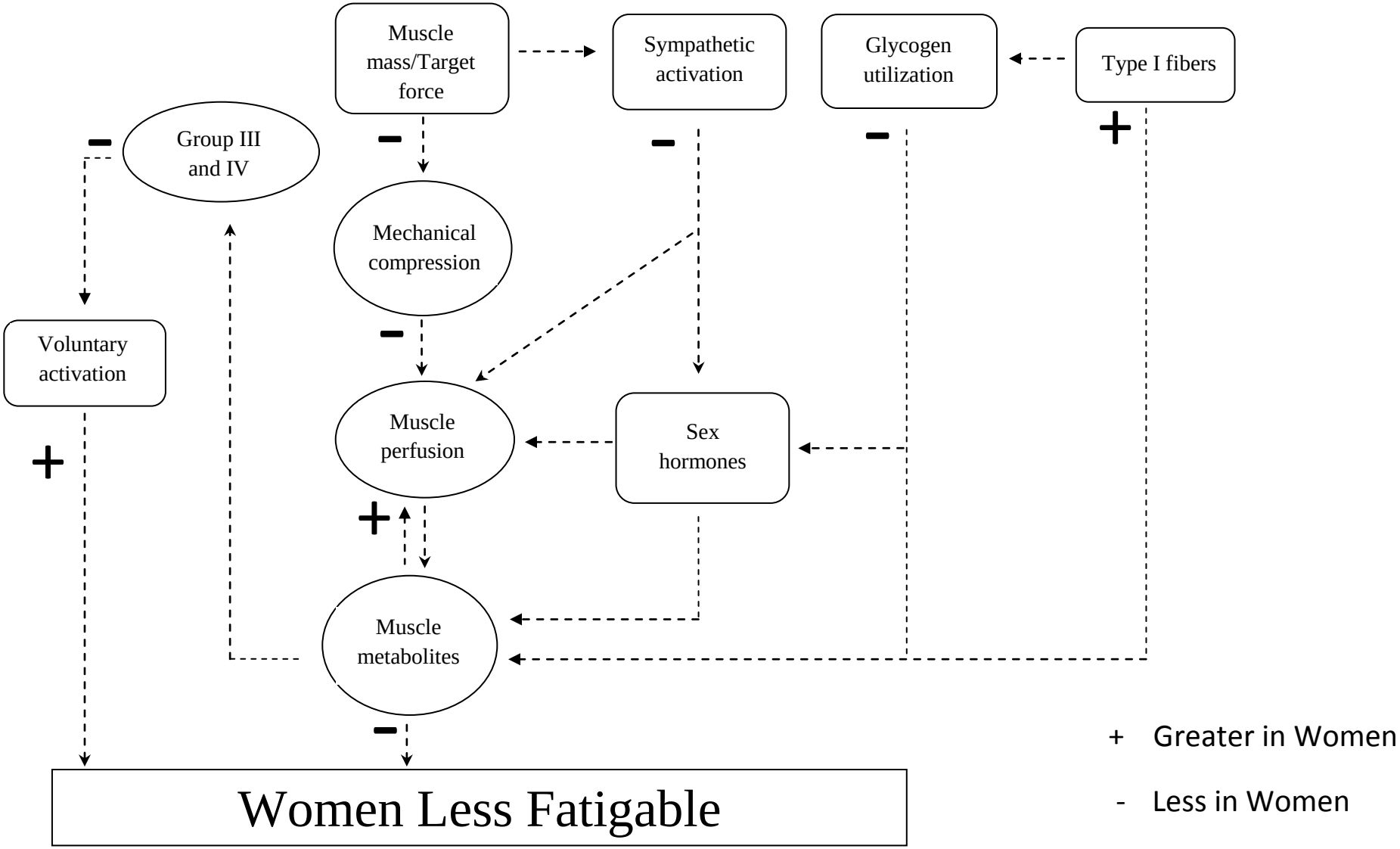

Figure 1.2. Sex Differences in Muscle Fatigue. Modified from Hunter *Exer Sci Sport Rev.* 2009. Model represents mechanisms of muscle fatigue for isometric contractions and is specific to the task and the strength of the mechanism will be dependent on the task.

Motor Output Variability

Modulation of motor output is important in both the presence of fatigue as well as stressful conditions. The ability to produce a target force and maintain a steady contraction requires the optimal recruitment and discharge frequency of motor units. Each motor unit exerts force which depends on the rate at which the motoneuron discharges action potentials (Thomas et al., 1991; Macefield et al., 1996). The force exerted by the motor unit will fluctuate about an average value when the discharge rate is less than that required for a fused tetanus. Because the discharge rates during voluntary contractions rarely achieve tetanic rates, the muscle will produce force fluctuations due to the submaximal activation of many motor units (Taylor et al., 2003). The potential mechanisms that may influence the motor output from a population of motor units are 1) organization of the motor-unit pool; 2) recruitment and rate-coding (discharge rate) properties of the motor units and 3) activation patterns of the motor-unit population (Taylor et al., 2003). One mechanism that appears to have the most influence on force fluctuations is the discharge rate variability of the motor unit (Laidlaw et al., 2000; Patten & Kamen, 2000).

Steadiness during low-intensity contractions is an important determinant of motor function and is associated with performance of functional tasks (Marmon et al., 2011). This can be quantified as the magnitude of force fluctuations or (coefficient of variation, CV) and calculated as the standard deviation of the force signal normalized to the mean force. Several factors can influence the variability in motor output including the age and sex of the individual (Noteboom et al., 2001b; Christou et al., 2004; Brown et al., 2010), the muscle group (Galganski et al., 1993; Graves et al., 2000; Tracy & Enoka, 2002), the

contraction type and intensity (Galganski et al., 1993; Keen et al., 1994; Laidlaw et al., 1999; Laidlaw et al., 2000) and the state of arousal (stress) (Noteboom et al., 2001a; Christou et al., 2004).

Force Fluctuations Increase with Fatigue

Variability of the force output is greater as the muscle becomes more fatigued (Cresswell & Loscher, 2000). As fatigue develops, the motoneuron pool receives less excitatory (or more inhibitory) afferent input due to an increase in feedback transmitted by chemically sensitive Type III and IV afferents (Bigland-Ritchie et al., 1986; Garland et al., 1994) and a reduction in feedback from stretch-sensitive afferents (Macefield et al., 1991; Duchateau et al., 2002). As the discharge rate of the motoneuron is reduced, the discharge rate will become more variable and greater synchronization of motor units (Holtermann et al., 2009) (which is likely to be greater among motor units with smaller and slower twitches) will occur (Schmied et al., 1993; Schmied et al., 1994). Additionally, coactivation of the antagonist muscle will lead to greater force fluctuations during a fatiguing contraction (Vallbo & Wessberg, 1993; Spiegel et al., 1996).

Sex Differences in Force Fluctuations

Differences in motor output variability between populations, muscle group and state of arousal are generally greater during low-intensity contractions when Type I (slow) motor units are predominantly recruited (Enoka et al., 2003; Christou et al., 2004; Brown et al., 2010). Women for example, who are usually weaker and possess a greater proportion of Type I fibers (Simoneau & Bouchard, 1989; Roepstorff et al., 2006) were less steady (greater force fluctuations) than men for low and high-intensity contractions, but the greatest sex difference was at the very low-intensity contractions (Brown et al.,

2010). The reason for the sex difference in force fluctuations is unknown and is further explored in these studies.

Section II. Acute Stress on Motor Performance

Physiological Response to Stress

Stress is defined as a state in which homeostasis (maintaining equilibrium or balance) is threatened or perceived to be so (Chrousos, 2009). A stressor is the internal or external event that causes the disruption of homeostasis (Chrousos, 2009). The stress response is mediated by the stress system both centrally and peripherally. Heightened physiological responses to stress are important determinants of health (Kajantie & Phillips, 2006), potentially suppress the immune response (Cohen & Herbert, 1996) and increase vulnerability to stress/anxiety disorders and musculoskeletal disorders (Holden, 2005; Passatore & Roatta, 2006).

The central control stations for the stress response located in the hypothalamus and the brainstem include the paravocellular corticotrophin releasing hormone (CRH) and arginine-vasopressin neurons. These neurons are located in the paraventricular nucleus of the hypothalamus in the brain and the locus coeruleus-norepinephrine system (LC/NE) (central sympathetic nervous system) located within the brainstem (Chrousos, 1992; Tsigos & Chrousos, 1994). The hypothalamic-pituitary-adrenal (HPA) axis together with the efferent sympathetic/adrenomedullary system, represent the effector limbs, via which the brain influences all body organs during exposure to threating stimuli (Tsigos & Chrousos, 2002). Figure 1.3 demonstrates the different actions of the hypothalamus, sympathetic nervous system and LC/NE system with the onset of acute stress.

CRH and CRH receptors are also found in many extrahypothalamic sites of the brain such as the limbic system, basal forebrain and the LC/NE system in the brain stem and spinal cord (Chrousos, 1998). CRH is known to completely initiate and coordinate behavioral and peripheral responses, which included characteristic stress behaviors and activation of the pituitary-adrenal axis and sympathetic nervous system. However, the paraventricular nucleus in the hypothalamus is the primary site for integration and regulation of glucocorticoid release as well as sympathoadrenal activation (Carvalho-Netto et al., 2011). CRH along with arginine-vasopressin penetrates the median eminence of the hypothalamus and stimulates the production of adrenocorticotropic hormone (ACTH) in the anterior pituitary by activating Pro-opiomelanocortin (POMC). ACTH will stimulate the release of glucocorticoids (cortisol in humans) from the adrenal cortex (Figure 1.3) (McEwen, 2000).

Glucocorticoids, cortisol in humans, have a variety of different effects in target systems throughout the organism, with one of the major effects of increasing the availability of energy substrates in different parts of the body, and allowing for optimal adaptations to changing demands of the environment (Lupien et al., 2007). As prolonged increases in circulating cortisol may increase the vulnerability to immunosuppression, and to autoimmune related and metabolic disorders it is essential that the termination of cortisol release is done appropriately. Circulating cortisol is regulated by a negative feedback system such that it will inhibit the synthesis and release of more cortisol at the anterior pituitary and hypothalamus (Figure 1.3) (Kirschbaum & Hellhammer, 1989).

The sympathetic nervous system, otherwise known as the fight or flight system, is a division of the autonomic nervous system and is involved in making sudden central and

peripheral physiological adjustments in the presence of threat or danger. This includes maintaining cardiovascular and regulatory dynamics, fluid and electrolyte homeostasis, energy balance, immune system operations, and many other functions (Powley, 2003). There is a hierarchical organization of the sympathetic nervous system into pre- and postganglionic levels. The cell bodies of the preganglionic neurons lie within the central nervous system, specifically in the brain stem and spinal cord. The sympathetic preganglionic neurons occupy the intermediolateral nucleus, a nearly continuous columnar grouping of cells running longitudinally through much of the spinal cord in the lateral horn of the gray matter in the spinal column (Powley, 2003). The axon of a preganglionic neuron typically exits from the segment in which its soma is located. Many of the preganglionic axons are lightly myelinated and separate from the ventral root to project to the appropriate peripheral ganglion.

Sympathetic postganglionic neurons are found in two distinct types of ganglia: paravertebral and prevertebral. As prevertebral ganglion innervate the gastrointestinal tract, the stomach and parts of the foregut, the paravertebral ganglion innervate the limbs, trunk, thorax and head (Figure 1.3) (Powley, 2003). The increase in sympathetic activity will activate the release of epinephrine and some norepinephrine from the adrenal medulla (sympathoadrenal system), and norepinephrine is released from nerve cells at other effector organs targeting mainly α and β receptors, except for the sweat glands which are muscarinic (Powley, 2003). In general, with the onset of stress a cascade of events occur including an increase in heart rate and blood pressure and blood vessels to the muscle dilate which will increase the flow of oxygen and energy. Simultaneously, the blood vessels in the gastrointestinal tract and skin constrict, reducing flow through these

organs and making more blood available to be shunted to skeletal muscle. These effects are mediated by activation of the sympathetic nervous system in conjunction with a withdrawal from the parasympathetic nervous system in the attempt for the individual to prepare an appropriate response to the stressor.

One of the important effects from increased sympathetic activity is changes in distribution of blood flow. Norepinephrine can increase the sympathetic outflow and alter blood perfusion. For example, norepinephrine will bind to the α-adrenergic receptors (Wallin et al., 1981) on the smooth muscle of the arterioles resulting in vasoconstriction and reduced blood flow to the muscle. Activation of the sympathetic neurons innervating nonactive muscle is believed to play an important role in the redistribution of blood flow to active muscle and the regulation of arterial pressure during exercise and mental stress (Callister et al., 1992; Callister et al., 1994). The sympathetic nervous system activity in the muscle can be directly measured by inserting a microelectrode in a peripheral nerve to record the sympathetic activity (Seals, 2006). Measurements of muscle sympathetic nerve activity (MSNA), an index of sympathoexcitation, demonstrate an increase in the bursting activity in the peroneal nerve when exposed to a mental stress and appears to be dependent on the intensity of the stressor (Callister et al., 1992). In other words, vasoconstriction was greater and blood flow reduced in the lower leg in the presence of mental stress.

Alternatively, mental stress is known to induce a vasodilator response (decrease in MSNA) and increase forearm blood flow (Halliwill et al., 1997). There has been controversy to the suggestion of vasodilator (cholingeric) nerves to the skeletal muscle (Joyner & Dietz, 2003). Cholinergic innervation is demonstrated in animals (Folkow et

al., 1948), but evidence has only been indirect in humans and in question over the past several years. Although there is some evidence of atropine induced vasodilation, mostly by local release of acetylcholine from endothelial cells and a rise in circulating epinephrine which can evoke β_2 – mediated vasodilation (in part because of NO release) (Joyner & Casey, 2009), it is now fairly accepted that the vasodilation response and increase in forearm blood flow is mostly mediated by nitric oxide (NO) (Joyner & Dietz, 2003).

Thus, there may be a dissociation of altered blood perfusion between the upper and lower limb muscles in the presence of mental stress. Where vasoconstriction and a decrease in blood flow occur in the lower limb, vasodilation and an increase in blood flow occur in the upper limb. This may be dependent of the type and intensity of the stressor or the response to stress may be muscle dependent. Additionally, in the presence of mental stress and exercise with the upper limb muscles, the balance of vasoconstriction and vasodilation and ultimately the blood perfusion to the muscle is likely to change but these relationships are not yet established.

Although not directly associated with an increase in sympathetic activation, serotonin is important for the central stress response. Serotonin is released from the dorsal raphe nucleus (brainstem) and is involved in the regulation of emotion, mood, sleep and aggression, and plays an important role in psychiatric diagnoses such as depression and PTSD (Krystal & Neumeister, 2009). Serotonin plays a role in regulation of the HPA axis as the release of serotonin will enhance the negative feedback control of cortisol on the HPA axis, as a biological mechanism for stress adaptation (Nuller & Ostroumova, 1980; Van Praag HM, 2004). Alternatively, dysfunctional serotonin activity

may deteriorate HPA function and reduce stress adaptation in animals (Seckl & Fink, 1991). Serotonin is also a potent neuromodulator of motoneuron excitability in the spinal cord and is important in modulating motor control (Forrest et al., 1996; Heckman et al., 2003).

Furthermore, there are sex differences in the stress response (Carvalho-Netto et al., 2011). Sex-specific hormones, i.e. estrogen, influence the synthesis of neuromodulators and stress hormones which may result in sex differences in sympathetic tone. For example, MSNA appears to be higher in young men compared with young women at rest (Ng et al., 1993), and also increases more for men compared with women during a static exercise (Ettinger et al., 1996), but demonstrates similar changes when exposed to mental stress (Jones et al., 1996; Carter & Ray, 2009). Clinically, the sex difference in sympathetic activation with stress is important because of the implications in the vulnerability of numerous disorders with known sex differences in incidence, such as anxiety (Carvalho-Netto et al., 2011), cardiovascular (Herd, 1991) and musculoskeletal disorders (Krantz et al., 2004; Wijnhoven et al., 2006).

In conclusion, the neuromodulators and hormones (norepinephrine, serotonin, epinephrine and cortisol) that are released with activation of the sympathetic nervous system and neuroendocrine system (Figure 1.3) impact cognitive (Lupien et al., 2007), cardiovascular (Herd, 1991) and motor functions (Lupien et al., 2007; Valentino & Van Bockstaele, 2008). Physiological manifestations of acute stress exposure and the fight-or-flight response ultimately lead to increased blood pressure, heart rate and cardiac output, changes in blood flow, increased sweating, and hormonal responses (McEwen, 2000; Kajantie & Phillips, 2006; Gerson et al., 2009) for an adequate and appropriate response

to the acute stressor. How stress can influence muscle fatigue and motor control is not well understood and will be discussed in the next section.

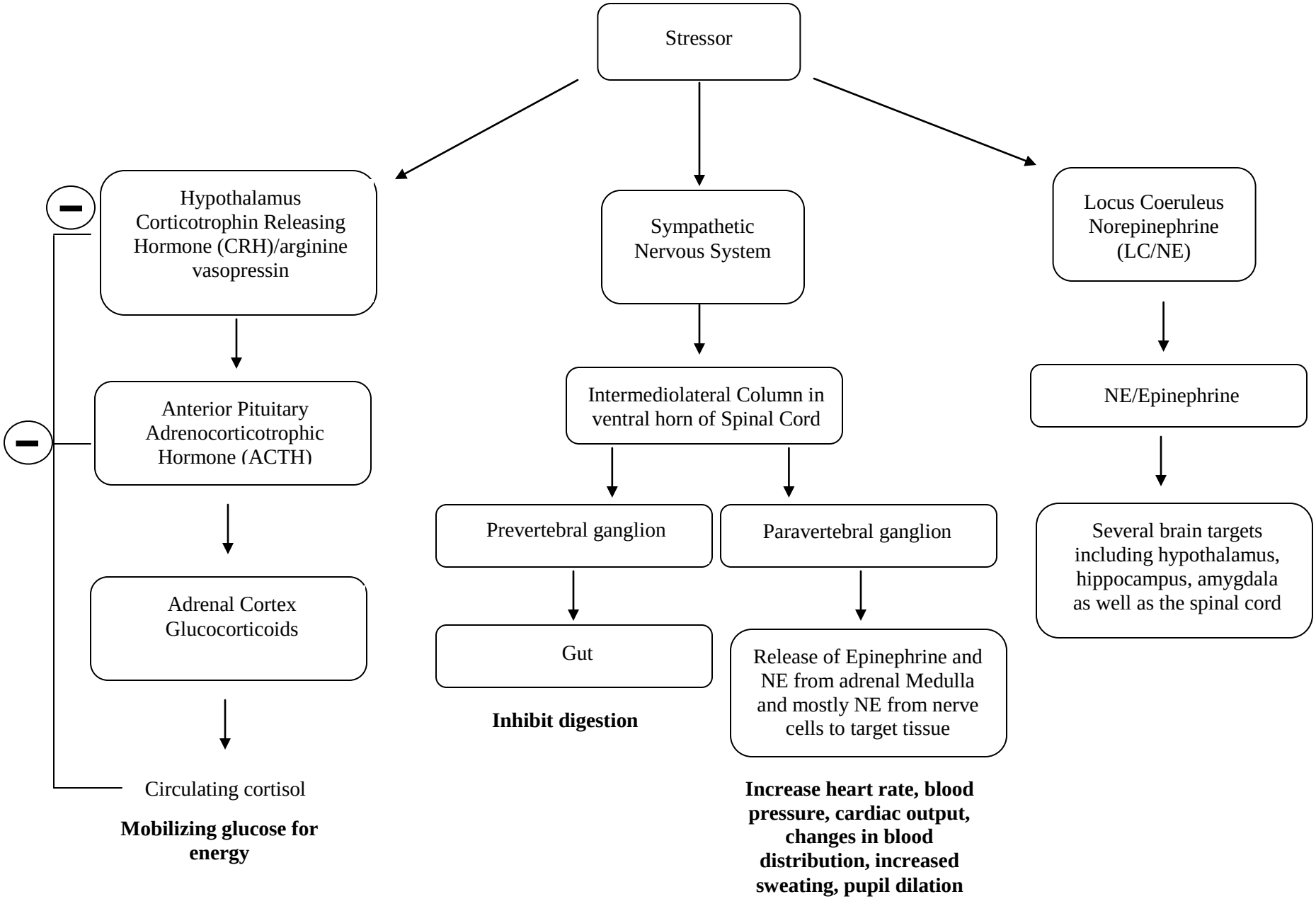

Figure 1.3. Acute Stress Response. Modified from Powley ed. *Fundamental Neuroscience* (2003) and Tsigos & Chrousos. *Journal of Psychosomatic*

Acute Stress, Muscle Fatigue and Motor Output Variability

Psychosocial stress can impair motor fatigue and increase motor output variability. When young adults, for example, are exposed to an acute stressor, muscle fatigability is greater (Yoon et al., 2009) and steadiness is reduced (difficult mental math task and noxious electrical stimulation) (Noteboom et al., 2001b; Christou et al., 2004) for low-intensity contractions (Yoon et al., 2009). The influence of stress appears to be more prominent in women than men and individuals with higher trait (general) anxiety (Noteboom et al., 2001a; Mottram et al., 2006; Yoon et al., 2009), although the mechanisms are unknown. Previous work has demonstrated that in the presence of electrical stimulation (as a stressor), men and women will become less steady for low-intensity contractions with the hand muscles and the reduction in steadiness was greater in the women (Noteboom et al., 2001b; Christou et al., 2004). Because the studies in this dissertation use stimulation techniques (both cortical and brachial plexus stimulation) to quantify mechanisms of muscle fatigue, the first aim was to confirm that the cortical and brachial plexus stimulation did not alter muscle fatigue or steadiness for the motor tasks performed in this study.

Sympathetic activation is well known to support motor function, but in the presence of stress, the sympathomedullary outflow (peripheral) (Figure 1.3) can result in excessive and inappropriate actions on motor control (Roatta et al., 2002; Passatore & Roatta, 2006; Roatta et al., 2008). Sympathetic activation exerts a number of actions at the muscular level, such as vasoconstriction (α_1) to skeletal muscles (Thomas & Segal, 2004), vasodilation (β_2) to skeletal muscles (Joyner & Dietz, 2003), modulation of contractility of fibers from different motor unit types (Bowman, 1980) and modulation of

the discharge of numerous receptors (in particular muscle spindles, which carry afferent feedback to the muscle for adequate motor control) (Roatta et al., 2002; Hellstrom et al., 2005). Because the stress-response is also centrally mediated (HPA axis and supraspinal circuitry), (Figure 1.3) stress-induced muscle fatigability may also be due to differences in output from the motor cortex (supraspinal fatigue). Furthermore, an upregulation of neuromodulators due to increased sympathetic activation may affect synaptic activity at the spinal cord level by increasing the input-output gain of the motoneurons and alter motor output (Heckman et al., 2003). Therefore, the stress-induced increase in sympathetic activation may be altering fatigability of the muscle and motor output variability by alterations in motor control by both peripheral and central mechanisms. Some of these mechanisms will be explored in Chapters III and IV.

Acute Stress and Muscle Fatigability

Acute stress can increase the fatigability of the muscle for healthy young men and women. For example, time to failure of a low-intensity fatiguing contraction with the elbow flexor muscles was reduced in men and women when performing a difficult mental-math task (cognitive stressor). The stress-induced reduction in time to failure was in part explained by the initial strength of the individual (Yoon et al., 2009). The greater fatigue was more pronounced in weaker subjects (usually women). Specifically, women had a 27% reduction and men had a 9% reduction in time to failure when exposed to the cognitive stressor. The greater fatigability was also accompanied by an increase in indices of sympathetic activation (mean arterial pressure) (Yoon et al., 2009). In the Yoon et al., (2009) study, cardiovascular measures were not direct predictors of the change in time to task failure for women; however, increased sympathetic activity may

have been involved and secondary to the initial strength of the subject. Possible explanations include: 1) altering skeletal muscle blood flow (Joyner & Halliwill, 2000; Thomas & Segal, 2004); and 2) reducing force and relaxation times of slow-twitch Type I fibers while potentiating force of fast twitch Type II fibers (Passatore & Roatta, 2006; Roatta et al., 2008).

The characteristics of the contractile properties, twitch amplitude and peak relaxation rates for example, can be associated with absolute strength (Hunter et al., 2006a) and blood perfusion to the muscle is associated with strength of the contraction force (Hunter et al., 2006b). Therefore, the association of initial strength and change in time to task failure suggests that either of these mechanisms (differences in blood perfusion or differential activation of contractile properties) may be contributing to the greater fatigability in women when exposed to a stressor (Yoon et al., 2009). This is because both perfusion of the muscle and its contractile force can be altered with an increase in sympathetic activation. The relative changes in blood flow due to stress-induced sympathetic activity could have influenced perfusion more so in women who exerted lower target forces and who may have had greater muscle perfusion than the stronger men during control conditions. A balance exists between vasoconstriction and vasodilation within the arterioles supplying the muscles during combined stress and exercise as vasoconstriction is blunted, but not eliminated, with static exercise in human subjects (Joyner & Casey, 2009). The majority of the vasodilation effects are from endothelium releasing factors such as nitric oxide (Joyner & Dietz, 2003) and are important during static contractions. Although the interactions are not fully understood, the balance of vasoconstriction and vasodilation with stress-induced sympathetic

activation could potentially alter the net perfusion of blood to the active muscle in women more than men during a low-intensity fatiguing contraction when exposed to a stressful task. That is, there is a greater opportunity for modulation of blood perfusion in women who are weaker because at the start of a sustained contraction at 20% of MVC, women are more likely to have a perfused muscle relative to men who are stronger.

An alternative explanation for the reduced time to failure of the women is altered contractility of Type I and Type II skeletal muscle fibers when sympathetic activation is greater (Passatore & Roatta, 2006). Accordingly, contractile force can be potentiated in Type II fast-twitch fibers but weakened in Type I slow-twitch fibers in animal and human muscle (Bowman, 1980; Roatta et al., 2008). The weakening action appears to be due to a potentiation of Ca^{2+} reuptake into the sarcoplasmic reticulum, which reduces the permanence of Ca^{2+} in the cytoplasm and results in a shortened twitch response (Roatta et al., 2008). In subtetanic contractions, this effect lowers the extent of twitch fusion and decreases the average force as reported for electrical contractions in response to intravenous injection of adrenaline (Marsden & Meadows, 1970; Bowman, 1980). Women demonstrate slower rates of muscle relaxation before fatigue for the elbow flexor and knee extensor muscles (Hunter et al., 2006a; Wust et al., 2008), which is consistent with a larger proportional area of slow twitch fibers in the muscles of women compared with men (Simoneau & Bouchard, 1989; Jaworowski et al., 2002). Women therefore, who appear to have a greater proportion of Type I fibers, may have greater reductions in time to failure in the presence of stress because of the weakening effects of sympathetic activation on Type I fibers.

The sex differences in fatigability can partly be explained by the absolute strength of the individual (21%) (Yoon et al., 2009) but may also be caused by differences in central activation in men and women. The stress response is also centrally mediated and there are sex differences in brain activation patterns during stressful cognitive tasks (Wang et al., 2007) and motor tasks (Wong et al., 2007). Additionally, descending command and synaptic input to the motor neuron pool is altered during tasks that require more attention (Johansen-Berg & Matthews, 2002). For example, changing the gain of the visual feedback signal during a target matching task increases the mental attentiveness (cognitive load) provided by the subject. The increased cognitive load resulted in a briefer time to failure for a low-intensity contraction with the elbow flexor muscles for women but not men (Mottram et al., 2006). This was related to a decline in discharge rates of the motor units in women during the high gain condition and a lower EMG bursting rate at the end of the task compared with men. These findings suggest that the increase in sympathetic activation induced by the cognitive load may have altered the motoneuronal output in women, potentially from greater inhibition by spinal or supraspinal sources, leading to greater fatigability for the women. Based on previous findings therefore, the aim of the second study is to investigate the mechanisms that contribute to the increased muscle fatigability when exposed to a cognitive stressor; specifically, fatigue originating from supraspinal sources and differences of the peak relaxation rates (a contractile property) will be investigated.

Mechanisms of Altered Motor Output Variability when Exposed to a Cognitive Stressor and Fatigue

When exposed to a stressor, men and women demonstrate greater force fluctuations for very low-intensity contractions (i.e. 4N and 2% of MVC) with the hand muscles (Noteboom et al., 2001b; Christou et al., 2004). The increase in force fluctuations however, were greater in women compared with men. The reason for the sex difference in force fluctuations with exposure to stress is unknown but may be due to greater motor unit discharge behaviors and activation of the muscle (surface EMG) when exposed to stress. Discharge rates of motor units and neural activation of the upper trapezius muscle were shown to be greater in resting conditions when exposed to an acute stress in women (Larsson et al., 1995; Lundberg, 2002; Stephenson & Maluf, 2010). One possibility is that women demonstrate greater amplitudes of motor output variability because they perceive the stimuli as more stressful than the men. Although no relationship has been established, previous studies indicate that women have greater perceptions of stress to similar stimuli (Jones et al., 2002; Lowery et al., 2003; Christou et al., 2004), although not always (Noteboom et al., 2001a). Additionally, women appear to have greater heart rates in the presence of stress compared with men (Noteboom et al., 2001b; Yoon et al., 2009), demonstrating greater activation of the sympathetic nervous system.

The stress-induced sympathetic activation may be altering motor output by interfering with proprioceptive information and up-regulating the release of neuromodulators in the central nervous system more for the women compared with men. In animal studies, artificial stimulation of the sympathetic nerve depresses muscle spindle

sensitivity (Matsuo et al., 1995; Roatta et al., 2002; Hellstrom et al., 2005). In the rat masseter muscle, sympathetic activation induced rapid inhibition (~0.5 s) of muscle spindle activity (Matsuo et al., 1995) that may have resulted from modulation of fusimotor drive from increased calcium reuptake that can inhibit afferent discharge (Roatta et al., 2002). Furthermore, sympathetic nerve activation (Hellstrom et al., 2005) and administration of a calcium antagonist have been shown to inhibit afferent discharge (Fischer & Schafer, 2002).

Sympathetic activation releases various neuromodulators that alter the membrane potential of motoneurons. Serotonin and norepinephrine can alter the synaptic input at the motoneuron in the ventral root of the spinal cord (Heckmann et al., 2005). In the presence of these monoamines, spinal motoneurons can depolarize the membrane potential towards voltage threshold which can modulate the input-output gain of the motoneurons and alter the recruitment and rate coding of motor units (Heckman et al., 2008). In turn, this may alter force fluctuations during a low-intensity contraction. The altered synaptic input at the motoneuron which can be modulated by physiological arousal, may interfere with modulation of force output (Marmon & Enoka, 2011), although it is currently unknown if the altered synaptic input would impair or enhance motor performance.

Consequently, it appears that sympathetic activation may modulate motor output in the presence of stress. Consistent with women perceiving stimuli as more stressful and having elevated heart rates in the presence of stress compared with men, women appear to modulate motor output differently, resulting in reductions in steadiness for low-intensity contractions in the presence of a stressor.

Muscle fatigue can also alter motor output variability by altering the synaptic input at the motoneuron (Cresswell & Loscher, 2000). Alterations in motor control due to fatigue that potentially contribute to increased force fluctuations include; 1) increased synchronization of motor units to the active muscle (Holtermann et al., 2009) (which is greater among motor units with smaller and slower twitches) (Schmied et al., 1993; Schmied et al., 1994), 2) coactivation during the motor task (Vallbo & Wessberg, 1993; Spiegel et al., 1996) and 3) reduced afferent feedback to motoneurons (Macefield et al., 1991) which could increase the variability in the discharge rate of the motoneuron (Holtermann et al., 2009). It has been recently demonstrated that increases in force fluctuations for a low-intensity (20% of MVC) fatiguing contraction when exposed to a cognitive stressor was similar for young men and women (Yoon et al., 2009). Whether cognitive stress and fatigue influence the sex differences in force fluctuations for very low-intensity (5% of MVC) contractions, where sex differences are the greatest and potentially most important for functional performance (Marmon et al., 2011), is unknown. Therefore, the third study will investigate the sex differences in force fluctuations for a very low-intensity contraction (5% of MVC) before and after a fatiguing contraction in the presence and absence of a cognitive stressor.

In summary, the first three studies of this dissertation attempt to understand how an acute stress can influence motor performance in healthy young men and women. Physiological responses to acute stress are important determinants of health (Kajantie & Phillips, 2006). The impact of acute stress during low-intensity fatiguing motor tasks that are commonly performed during daily tasks, have important clinical implications in vulnerable populations. As such, exposure to an acute *traumatic* stress results in long-

term physiological adaptations within the stress systems. Posttraumatic Stress Disorder (PTSD) is a chronic stress disorder that can occur after exposure to an acute traumatic event. The adaptations that occur within the sympathetic nervous and neuroendocrine systems in individuals with PTSD may influence the neuromuscular system and alter motor performance in this clinical population. This will be discussed in the next section.

Section III. PTSD and Motor Performance

Posttraumatic Stress Disorder Characteristics

PTSD is caused by acute exposure to a traumatic event involving the threat of death or serious injury that leads to a reaction of intense fear, helplessness or horror (APA, 2000). PTSD involves a) re-experiencing the traumatic event, b) avoidance of stimuli and emotional numbing and c) symptoms of hyperarousal (APA, 2000). Veterans of war often have a high prevalence of combat or military related PTSD with a lifetime prevalence in Iraq and Afghanistan combat veterans of 15% (Schnurr, 2010). The frequency and intensity of exposure to combat experiences is strongly associated with the risk of chronic PTSD (APA, 2000) related cognitive impairment (Kaylor et al., 1987).

Consequently, this population demonstrates distinct pathological adaptations to chronic stress which have significant clinical implications in overall health and function. For example, findings from the National Vietnam Veterans Readjustment Study (Kulka, 1990) showed that Vietnam veterans with PTSD had greater work impairment and unemployment, poorer physical health, greater physical limitations and more medical utilization than those without PTSD. The cost of PTSD to society is substantial as an

estimated $3 billion loss of productivity annually is due to PTSD (Kessler, 2000). In addition, individuals with PTSD have significant reductions in physical activity levels (de Assis et al., 2008) and report greater symptoms of musculoskeletal pain and disorders (Dunn et al., 2011). Therefore, symptoms of PTSD and the pathophysiological changes appear to disrupt daily function and may have severe consequences on work-related tasks necessary for job performance and military action.

Neurobiology of PTSD

PTSD symptoms are presently hypothesized to reflect either pathological changes in stress-response systems or failure of neurobiological systems to adapt to extreme stressors (Benedek, 2011). Individuals with PTSD demonstrate significant alterations in their stress-regulation systems. Patients with PTSD exhibit chronic hyperactivation of the central nervous system and sympathetic nervous system leading to greater basal elevations of monoamines, i.e. norepinephrine, epinephrine and serotonin (Southwick et al., 1999) and a dysregulation of the HPA axis resulting in lower basal cortisol levels (Yehuda, 2005), although not always (Liberzon et al., 1999). PTSD also involves alterations in cortical structures that are involved in the stress response, but that are also important in anxiety and the fear response. For example, a dysregulation in the hypothalamus, prefrontal cortex (Bremner, 2007b; Weinberg et al., 2011), hippocampus (Bremner, 2007a), amygdala (Rogers et al., 2009) and brainstem nuclei (Valentino & Van Bockstaele, 2008) have been shown in patients with PTSD. The cortical changes are closely linked with the symptomology of PTSD described previously and demonstrate the complex integrative nature of this disorder.

Autonomic control is altered in those with PTSD. Patients with PTSD demonstrate greater baseline levels of anxiety, heart rate and blood pressure (Bedi & Arora, 2007; O'Donnell et al., 2007). The elevated tonic and phasic levels of heart rate that occur weeks and months after exposure to the trauma are shown to be predictive of PTSD (O'Donnell et al., 2007). Elevated heart rates and blood pressures are known to be indices of sympathetic activation (Ettinger et al., 1996) and are related to the greater plasma levels of monoamines that are elevated in patients with PTSD (Southwick et al., 1999; Bedi & Arora, 2007). This is suggestive that basal levels of sympathetic activity are higher in those with PTSD.

Additionally, war veterans with PTSD demonstrate greater autonomic responses in the presence of stressful stimuli. For example, combat veterans with PTSD exhibit significantly greater heart rate and blood pressure responses to audiovisual presentations of combat situations than non-psychiatric subjects with and without combat experience (Blanchard et al., 1982; Malloy et al., 1983; Blanchard, 1986). Veterans with PTSD also exhibit greater frontalis EMG and skin conductance responses than asymptomatic combat veterans when imaging personally meaningful combat trauma situations (Pitman et al., 1987). Importantly, Mcfall et al. (1990) demonstrated that elevated heart rates and mean arterial pressure to combat films was consistent with the increased plasma catecholamines (epinephrine and norepinephrine). Together, these studies indicate that combat veterans with PTSD display greater physiological reactivity to diverse forms of combat-related cues than do patient and nonpatient controls.

PTSD, Acute stress and Motor Performance

The previous section indicated that acute levels of stress and increased sympathetic activation may be the cause of alterations in motor performance (increased muscle fatigability and reductions in steadiness). Those with PTSD demonstrate augmented levels of sympathetic nervous system activity, but how these adaptations may alter motor performance in veterans with PTSD is unknown. Reaction time to an auditory task was reported to be greater in individuals with PTSD (McFarlane et al., 1993) while other studies showed no differences in reaction time compared with healthy controls (Metzger et al., 1997; Galletly et al., 2001). The ability to maintain a steady contraction for brief and long durations of time is crucial for vocational tasks as well as military tasks, but the influence of PTSD on muscle fatigue and steadiness for low-intensity contractions is unknown. Therefore, the first purpose of the fourth study is to determine if veterans with PTSD will fatigue more quickly and demonstrate greater reductions in steadiness of target matching tasks during low-intensity contractions with the hand muscles when compared with control subjects.

Furthermore, individuals with PTSD have an augmented activation of their sympathetic nervous system at baseline and greater physiological responses to acute stressors than healthy adults. It is unknown if the abnormalities of the stress response demonstrated in individuals with PTSD (Malloy et al., 1983; Pitman et al., 1987; Blanchard et al., 1996) will affect fatigability of the muscle or the ability to maintain a steady contraction. Therefore, the second purpose of the fourth study is to determine if exposure to an acute cognitive stress will impair motor performance (increase muscle

fatigability and reductions in steadiness) for low-intensity contractions of the handgrip muscles in veterans with PTSD.

Importantly, a confounder of PTSD is the higher prevalence of pain (Geuze et al., 2007). One of the symptoms of PTSD, hyperarousal, was shown to be associated with pain intensity and together pain and hyperarousal lead to eventual pain avoidance and diminished daily functioning (Cho et al., 2011). Additionally, pain processing appears to be different in individuals with PTSD with greater activation of the hippocampus and decreased activation in the PFC and amygdala (Geuze et al., 2007) than those without PTSD. It is important to understand how chronic pain and perceptions of pain may influence motor performance. It is currently unknown if chronic pain will alter performance during motor fatiguing tasks, particularly in patients with PTSD. Therefore, the last study of this dissertation will quantify acute perceptions of pain and subjective ratings of chronic pain to determine if pain in PTSD is associated with any alterations found in motor performance.

Dissertation Aims and Hypotheses

Aim 1: To investigate stress-induced alterations in muscle fatigue and motor output variability of isometric contractions in *young healthy men and women*.

Sub aims:

1. To determine if supraspinal fatigue contributes to the sex differences in neuromuscular fatigability for a low-intensity contraction. *Hypothesis: Supraspinal fatigue will contribute to muscle fatigue in men and women similarly after a low-intensity fatiguing contraction with the elbow flexor muscles.*
2. To compare time to failure and motor output variability for a low-intensity fatiguing contraction of men and women in the presence and absence of cortical and electrical stimulation. *Hypothesis: Cortical and electrical stimulation, used to assess neural mechanisms of fatigue, are strong enough irritants (stressors) to alter the time to task failure and physiological adjustments during the fatiguing contraction in men and women.*
3. To determine the contribution of contractile properties and supraspinal fatigue to stress-induced changes in muscle fatigability in the upper limb muscles. *Hypothesis: Weaker adults (usually women) will have greater decrements in time to failure of a submaximal isometric fatiguing contraction with exposure to a cognitive stressor and this would is associated with slower rates of relaxation and greater supraspinal fatigue in the elbow flexor muscles.*
4. To determine the sex difference in force fluctuations for a very low-intensity contraction (5% of MVC) with the elbow flexor muscles before and after a fatiguing contraction in the presence and absence of a cognitive stressor. *Hypothesis: Women*

will have greater increases in force fluctuations during very low-intensity tasks than men when exposed to fatigue and an acute stressor.

<u>Aim 2:</u> To determine muscle fatigability and motor output variability of isometric contractions of <u>*war veterans with PTSD*</u> in the presence and absence of an acute stressor.

Sub aims:

1. To determine if veterans with PTSD fatigue more quickly and are less steady than control subjects during a low-intensity contraction with the handgrip muscles.
   Hypothesis: Veterans with PTSD will fatigue more quickly and be less steady than control subjects for a low-intensity fatiguing contraction with the hand muscles.
2. To determine if exposure to an acute cognitive stress will impair motor performance (increase muscle fatigability and reductions in steadiness) for low-intensity contractions of the handgrip muscles in veterans with PTSD.
   Hypothesis: Veterans with PTSD will have even greater impairments in motor performance when exposed to the acute stressor compared with healthy controls.

Chapter II

Supraspinal Fatigue is Similar in Men and Women for a Low-Intensity Fatiguing Contraction

SUMMARY

This study determined if supraspinal fatigue contributed to the sex difference in neuromuscular fatigability for a low-intensity fatiguing contraction. Because women have greater motor responses to arousal than men, we also examined whether cortical and motor nerve stimulation, techniques used to quantify central fatigue, would alter the sex difference in muscle fatigue. In Study 1, cortical stimulation was elicited during maximal voluntary contractions (MVC) before and after a submaximal isometric contraction at 20% MVC with the elbow flexor muscles in 29 young adults (20 ± 2.6 yrs., 14 men). In Study 2, 10 men and 10 women (19.1 ± 2.9 yrs.) performed a fatiguing contraction in the presence and absence of cortical and motor nerve stimulation. Study 1: Men had a briefer time to task failure than women ($P = 0.009$). Voluntary activation was reduced after the fatiguing contraction ($P < 0.001$) similarly for men and women. Motor evoked potential area and the EMG silent period increased similarly with fatigue for both sexes. Peak relaxation rates however were greater for men than women and were associated with time to task failure ($P < 0.05$). Force fluctuations, rating of perceived exertion (RPE), heart rate and mean arterial pressure increased at a greater rate for men than women during the fatiguing contraction ($P < 0.05$). Study 2: Time to task failure, force fluctuations and all other physiological variables assessed were similar for the control session and stimulation

session ($P > 0.05$) for both men and women. Supraspinal fatigue was similar for men and women after the low-force fatiguing contraction and the sex difference in muscle fatigue was associated with peripheral mechanisms. Furthermore, supraspinal fatigue can be quantified in both men and women without influencing motor performance.

INTRODUCTION

There are sex differences in muscle fatigue for both maximal and submaximal isometric tasks (Hunter, 2009). For example, when men and women perform a submaximal isometric fatiguing contraction at the same relative intensity, women can sustain the contraction for a longer duration than men most of the time for many muscle groups including the elbow flexors, finger flexors and knee extensors (Hunter, 2009). Similarly, women exhibit less of a reduction in maximal force than men during sustained and intermittent maximal contractions (Russ & Kent-Braun, 2003; Hunter et al., 2006a; Martin & Rattey, 2007).

Neural and muscular mechanisms contribute to muscle fatigue experienced by men and women (Russ & Kent-Braun, 2003; Hunter et al., 2006a; Yoon et al., 2007). The sex difference in muscle fatigue however and the contributing mechanisms are specific to the demands of the task and muscle groups involved (Hunter, 2009). By stimulating the motor nerve during maximal force contractions, reductions in voluntary activation (commonly known as central fatigue) can be assessed (Gandevia, 2001). Using motor nerve stimulation, central fatigue explained the sex difference in muscle fatigue during maximal fatiguing contractions of the dorsiflexor muscles (Russ & Kent-Braun, 2003) and the knee extensor muscles (Martin & Rattey, 2007). For repeated maximal contractions of the upper limb, supraspinal fatigue did not contribute to the sex difference

in muscle fatigue of the elbow flexor muscles (Hunter et al., 2006a). Supraspinal fatigue is a component of central fatigue and can be identified by stimulating at the motor cortex with transcranial magnetic stimulation (TMS) (Gandevia, 2001). Low-force fatiguing contractions however, can also elicit substantial central fatigue originating from supraspinal and spinal sources that was collectively similar in men and women (Yoon et al., 2007). This study therefore determined if there were sex differences in supraspinal fatigue elicited from low-force fatiguing contractions. As found during maximal strength fatiguing contractions of the upper extremity (Hunter et al., 2006a), we hypothesized supraspinal fatigue would contribute to muscle fatigue in men and women similarly after a low-intensity fatiguing contraction with the elbow flexor muscles.

The contribution of the neural mechanisms to the sex difference in performance of a low-intensity fatiguing contraction may depend on the state of arousal or mental attentiveness of men and women (Yoon et al., 2009). Women demonstrated greater reductions in time to failure (greater fatigability) than men when exposed to a cognitive stressor (mental math) during a low-intensity fatiguing contraction with the elbow flexor muscles (Yoon et al., 2009). Physical stressors are also used experimentally to effectively increase arousal in healthy adults but the stress response can differ between men and women (Kudielka & Kirschbaum, 2005). Repeated exposure to electrical stimulation for example, can evoke increased arousal and greater variability in motor output during isometric tasks particularly in women and individuals with high levels of trait anxiety (Noteboom et al., 2001a; Christou et al., 2004).

Electrical stimulation is also an important technique used to quantify neural mechanisms of muscle fatigue (14). Stimulating the motor nerve or muscle can elicit an

M wave (compound muscle action potential) and evoke a twitch or tetanic contraction that is subsequently used to calculate voluntary activation. TMS that is used to quantify supraspinal fatigue (Gandevia, 2001) is brief, but is also typically repeated throughout experimental protocols. Thus, these stimulation techniques potentially promote an increase in stress altering performance of a fatiguing contraction by different magnitudes in men and women. Electrical stimulation for example, used to assess voluntary activation of the quadriceps, resulted in a reduction of quadriceps force and electromyography (EMG) in men (Button D, 2008). Therefore, a second purpose of this study was to compare time to failure and motor output variability for a low-intensity fatiguing contraction of men and women in the presence and absence of cortical and electrical stimulation. We hypothesized that cortical and electrical stimulation, which are used to assess neural mechanisms of fatigue, could be strong enough irritants (stressors) to alter the time to task failure and physiological adjustments during the fatiguing contraction in men and women. To understand the physiological adjustments during the fatiguing contractions with and without stimulation in both men and women, we monitored muscle activation patterns using EMG, cardiovascular adjustments (mean arterial pressure and heart rate) and force fluctuations as a measure of motor output variability.

METHODS

Twenty-nine young adults [14 men (20.1 ± 1.9 yrs) and 15 women (19.9 ± 3.2 yrs)] volunteered to participate in a session that assessed the sex differences in supraspinal fatigue for a low-force fatiguing contraction with the elbow flexor muscles (study one). A subset of these subjects, 20 young adults [10 men (19.8 ± 2.2 yrs) and 10

women (20.3 ± 3.8 yrs)] participated in an additional experiment to assess the influence of stimulation on fatigability (study two). All subjects were healthy with no known cardiovascular or neurological disorders and they were naïve to the protocol. Each subject had low-to-moderate levels of trait anxiety (21 to 48) assessed with the State-Trait Anxiety Inventory (STAI) (Spielberger & Vagg, 1984) and reported no history or current mental pathology, including anxiety or depressive disorder. Before participation, each subject attended a familiarization session and provided written informed consent. All experiments were approved by the Marquette University Institutional Review Board.

The physical activity level of each subject was assessed by a questionnaire that estimated the relative kilocalorie expenditure per week (Kriska & Bennett, 1992). The day of the menstrual cycle on which the experimental protocols were performed was recorded for each female subject. The first day of menstruation was considered day one of the cycle. Hand dominance was estimated using the Edinburgh Handedness Inventory (Oldfield, 1971) with a score of 0.53 for men and 0.60 for women ($P = 0.52$) and a ratio of 1 indicated complete right-handedness.

All subjects participated in a familiarization session and then attended one experimental session for study one and an additional session for study two (counterbalanced among subjects). The experimental sessions were ≥7 days apart to perform a protocol that involved a fatiguing contraction with the elbow flexor muscles of the left arm. The familiarization session involved habituating the subject to the procedures including motor cortical and brachial plexus stimulation, performance of several trials of the maximal voluntary isometric contraction (MVC) task and the submaximal voluntary contraction task.

For each study, the experimental procedures involved performance of MVCs with elbow flexor muscles followed by a fatiguing contraction at 20% of MVC until task failure and recovery measures that are described in greater detail in the experimental protocol. For study one, subjects performed the experimental session with procedures that involved stimulation of the brachial plexus and motor cortex. For study two, subjects attended two sessions (1) a stimulation session as described above that involved using TMS to assess voluntary activation and corticomotor excitability and electrical stimulation of the brachial plexus (TMS followed by brachial plexus stimulation) and (2) a control session with no stimulation of the cortex or brachial plexus. The procedures were similar for the two sessions with the exception of the delivery of electrical and magnetic stimulation during the maximal contractions before and after the fatiguing task as well as during the submaximal fatiguing task.

Mechanical Recordings

Each subject was seated upright in an adjustable chair with their left arm slightly abducted. Their elbow rested comfortably on a padded support and the elbow joint was flexed to 90 degrees so that the forearm was horizontal to the ground. The shoulders were restrained by two nylon straps to minimize shoulder movement. The hand and forearm were placed in a modified rigid wrist-hand-thumb orthosis (Orthomerica, Newport Beach, CA) midway between pronation and supination and the force was directed upward when the elbow flexor muscles were activated. The forces exerted by the wrist in the vertical and horizontal directions were measured with a force transducer (Force-Moment Sensor, JR-3, Woodland, CA) that was mounted on a custom designed, adjustable support. The orthosis was fixed to the force transducer. The forces detected by the transducer were

recorded online by using a Power 1401 A-D converter and Spike 2 software [Cambridge Electronics Design (CED), Cambridge, UK]. The force exerted in the vertical direction was displayed on a 19-inch monitor located ~1.5 m in front of the subject and the force signal was digitized at 500 samples/s. Force fluctuations which typically increase during the fatiguing contraction (Cresswell & Loscher, 2000; Hunter & Enoka, 2001), were quantified as a measure of motor output variability. The amplitude of the force fluctuations was quantified as the coefficient of variation of the force (CV = SD/mean × 100) (Hunter & Enoka, 2001).

Electrical Recordings

As a global measure of muscle activation, EMG signals were recorded with bipolar surface electrodes (Ag-AgCl, 8-mm diameter; 16 mm between electrodes) that were placed over the long head of the biceps brachii, brachioradialis, and long head of the triceps brachii muscles. The bipolar electrode configuration was placed longitudinally over the muscle belly midway between the origin and insertion for each muscle, according to the European recommendations for surface electromyography (Hermens et al., 2000). Reference electrodes were placed on the lateral epicondyle of the elbow. The EMG signals were amplified (100x) and band-pass filtered (13–1,000 Hz) with Coulbourn modules (Coulbourn Instruments, Allentown, PA). The signal was displayed on an oscilloscope and recorded online via a Power 1401 A-D converter (CED). The EMG signals were digitized at 2000 samples/s.

Cardiovascular Measurements

To understand sex differences in cardiovascular adjustments (Gandevia & Hobbs, 1990; Hunter & Enoka, 2001), heart rate and mean arterial pressure (MAP) were monitored during the fatiguing contraction with an automated beat-by-beat, blood pressure monitor (Finapres 2300, Ohmeda, Madison, WI). The blood pressure cuff was placed around the middle finger of the relaxed right hand with the arm placed on a table adjacent to the subject at heart level. The blood pressure signal was recorded online to the computer at 500 samples/s.

Cognitive Assessment of Arousal (Study 2)

Cognitive levels of anxiety were assessed throughout the protocol using a visual analogue scale (VAS) (Johnson, 2001) and the state portion of the STAI questionnaire (Spielberger & Vagg, 1984). The VAS for anxiety involved a 10-cm line anchored at the far left by "not at all anxious" and at the far right by "very anxious". Each subject indicated their level of anxiety with a mark on the 10-cm line. They were instructed that anxiety was defined as the negative emotions regarding the immediate future (Christou et al., 2004). VAS for anxiety were recorded at 7 time points during the protocol: one immediately prior to being seated for the experimental session [T1], after performing voluntary contractions (and first bouts of stimulation during stimulation session) [T2], one after the 20 min rest [T3], immediately after the fatiguing contraction [T4]; and then 5, 10 and 20 min during recovery after fatiguing contraction [T5-T7] (Figure 2.1). The STAI-state questionnaire involved 20 statements that required a response on a four-point, Likert-type scale. Assessment of STAI was performed at baseline and at the start of the quiet rest (Figure 2.1).

Stimulation

Subjects were stimulated at the brachial plexus with electrical stimulation and at the motor cortex with TMS for study 1 and the stimulation session of study 2.

Brachial Plexus Stimulation. The brachial plexus was electrically stimulated to produce a maximal compound muscle action potential (maximum M wave: M_{max}) of the biceps brachii, brachioradialis and triceps brachii muscles. Stimulation intensity was increased until the M wave amplitude plateaued for all three muscles. The stimulation intensity ranged between 120 and 300 mA. A cathode was placed in the supraclavicular fossa and an anode on the acromion. A constant-current stimulator (model DS7AH, Digitimer, Welwyn Garden City, Hertforshire, UK) was used to deliver single stimuli (100 μs duration) to the brachial plexus.

Motor Cortex Stimulation. TMS stimulation was delivered via a round coil (13.5-cm outside diameter) over the vertex (Magstim 200, Magstim, Whitland, UK) to elicit motor-evoked potentials (MEPs) in biceps brachii, brachioradialis and triceps brachii muscles. The vertex of the motor cortex was identified and the scalp marked to ensure repeatability of coil placement throughout the protocol. The right cerebral hemisphere was stimulated by the direction of the current flow in the coil to preferentially activate the left limb. A single pulse was delivered over the motor cortex at an intensity (80-95% of maximum stimulator output) that produced a large MEP in the agonist biceps muscle (minimum amplitude of 50% of M_{max} during a brief MVC of the elbow flexor muscles but only a small MEP in the antagonist triceps muscle amplitude < 20 % of M_{max}) (Todd et al., 2004). TMS was delivered during voluntary contractions only.

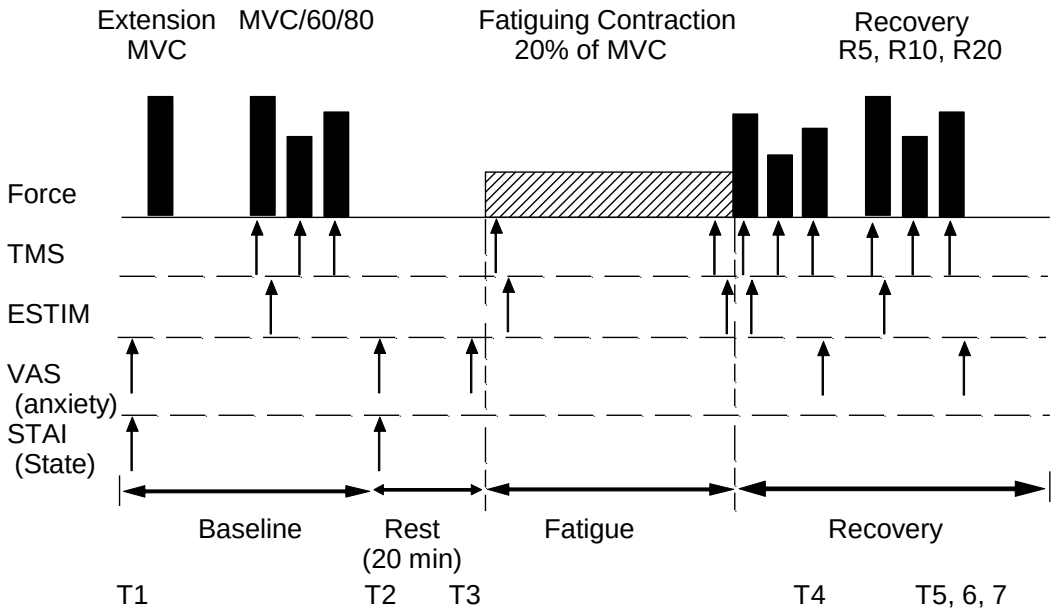

Figure 2.1: Experimental Protocol. The top panel shows the order of force tasks performed by each subject with the elbow flexor muscles. Maximal voluntary contraction (MVC) with the elbow extensors were performed followed by MVCs of the elbow flexor and brief contractions at 60 and 80% of MVC which were also performed during recovery of the fatiguing contraction (R5, R10 and R20 denotes recovery at 5, 10 and 20 minutes respectively). In the second and third rows, the arrows denote the time points that transcranial magnetic stimulation (TMS) and electrical stimulation of the brachial plexus (Estim) were delivered respectively during the stimulation session only. State-Trait Anxiety Inventory (STAI) questionnaire was assessed twice throughout the protocol. Please note that the schematic is not to scale for time or force.

Experimental Protocol

At the start of the session for study one and stimulation session for study two, optimal levels of stimulation intensities to the motor cortex and brachial plexus were determined and these levels remained constant throughout the rest of the protocol. All procedures thereafter were as follows and indicated in Figure 2.1:

1. *Maximal Voluntary Contractions (MVC).* Two MVC*s* of the elbow extensors were performed so that peak EMG values could be obtained to normalize the triceps EMG activity during the fatiguing contractions. No stimulation was delivered during the

elbow extensor contractions for either session. Four sets of brief contractions (2-3s) with the elbow flexor muscles were performed and separated by 2 minutes of rest to minimize fatigue. Each set involved performance of a MVC followed by contractions at 60% and 80% MVC. Within each set, the start of each contraction was separated by 3-4 s. If peak forces from two of the four MVC's trials were not within 5% of each other, additional trials were performed until this was accomplished. During the stimulation session, TMS was delivered during each contraction and brachial plexus stimulation was delivered during the MVCs only. During the control session all maximal and submaximal contractions were performed without the motor cortical or brachial plexus stimulation.

2. *Fatiguing Contraction.* A fatiguing contraction was performed with the elbow flexor muscles in each session at a target value of 20% MVC force (calculated from the peak MVC force). Each subject was required to match the vertical target force that was displayed on the monitor and encouraged to sustain the force for as long as possible. The fatiguing contraction was terminated when the force had declined by 10% of the target value for 2 out of 4 consecutive seconds. Task failure was detected automatically using a custom-designed program (Spike 2, CED) that monitored the force signal, and this time was recorded as the time to task failure. During the stimulation session, TMS and brachial plexus stimulation were delivered at the start and end of the fatiguing contraction (Figure 2.1). As an index of perceived effort, each subject verbally indicated their rating of perceived exertion (RPE) using the modified Borg 10-point scale at the start of the fatiguing contraction, every minute thereafter and at task failure (Borg, 1982). The scale was anchored so that 0 represented the resting state and 10 corresponded to the strongest

contraction that the arm muscles could perform. The RPE was recorded at the beginning of the contraction and every minute thereafter until task failure.

3. *Recovery Measures*. Torque measurements, voluntary activation and perceived levels of anxiety were assessed immediately upon task failure, and then at 5 min, 10 min and 20 min after termination of the fatiguing contraction (see Figure 2.1).

Data Analysis

The MVC force was quantified as the average value over a 0.5-s interval that was centered about the peak of the MVC. The torque for the MVC and submaximal contractions was calculated as the product of force and the distance between the elbow joint and the point at which the wrist was attached to the force transducer. The maximal EMG for each muscle was determined as the root mean squared (RMS) value over a 0.5-s interval about the same interval of the MVC torque measurement. The maximal EMG value for the biceps brachii, brachioradialis and triceps brachii was then used to normalize the RMS EMG values recorded during the fatiguing contraction for each respective muscle. The RMS EMG of the elbow flexor muscles and triceps brachii muscles and the fluctuations in force (CV of force) were quantified during the fatiguing contraction at the following time intervals: the first and last 30 s of task duration and 15 s either side of 25%, 50%, and 75% of time to failure.

Heart rate and MAP were recorded during the fatiguing contraction and analyzed by comparing ~15-s averages at 25% intervals. For each interval, the blood pressure signal was analyzed for the mean peaks [systolic blood pressure (SBP)], mean troughs [diastolic blood pressure (DBP)], and number of pulses per second (multiplied by 60 to

determine heart rate). MAP was calculated for each epoch with the following equation: MAP = DBP +1/3(SBP – DBP).

The amplitude of the superimposed twitch (SIT) elicited by TMS is reported as a percentage of the voluntary torque measured immediately prior to TMS (Gandevia, 2001). The SIT amplitude was also used to calculate voluntary activation. Voluntary activation was quantified by expressing the amplitude of the SIT (elicited by TMS) as a fraction of the estimated amplitude of the response evoked by the same stimulus at rest (estimated resting twitch, eRT). Because motor cortical and spinal cord excitability increase with activity a control resting twitch was not able to be achieved at rest, therefore the amplitude of the resting twitch was estimated rather than measured directly (Todd et al., 2003). During the sets of brief maximal and submaximal contractions (MVC followed by 60% and 80% MVC contractions) TMS was delivered and the resting twitch was estimated by extrapolation of the linear relation between the amplitude of the SIT and voluntary force. One regression analysis was performed for each set of brief contractions. The y-intercept was taken as the estimated amplitude of the resting twitch evoked by TMS. The amplitude of the estimated resting twitch can be accurately determined from three data points in fresh or fatigued muscle when the contractions are greater than 50% MVC (Todd et al., 2003). Voluntary activation (%) was calculated as a percentage measured by cortical stimulation [(1-SIT/eRT) x 100] (Todd et al., 2003). Data points were excluded (5.5%) for subjects at different time points when the regression of the estimated twitch was $r < 0.9$.

Contractile properties of the elbow flexor muscle fibers were also assessed. The amplitude of the estimated resting twitch was used as an index of the force generating

capacity of the elbow flexor muscles and the fall of the force after cortical stimulus was used to determine the peak relaxation rate of the whole muscle (Todd et al., 2007). Peak relaxation rates were determined during each MVC by calculating the steepest falling of the force during the EMG silence immediately following TMS (Todd et al., 2007). This was determined as the highest negative derivative of the torque for an interval of 10 ms between two cursors placed either side of the fall in force during the silent period. The steepest rate of force decline was normalized to the total force (MVC plus superimposed twitch) prior to the silent period (Todd et al., 2007).

The amplitude and area of MEPs and M wave were measured between two cursors placed at the start and end of the waveform for the biceps and triceps muscles. As the MEP amplitude and area showed similar changes, only MEP area is reported. M waves were elicited after each MEP, but because there were no changes in M wave, the MEP is represented as a percent change from their baseline values. When TMS is delivered during a voluntary contraction, the MEP is followed by a period of near-silence in the EMG (Inghilleri et al., 1993), lasting more than 200 ms with a high intensity stimulus. The silent period was measured as the interval from the stimulus to the resumption of continuous EMG. Voluntary torque was quantified by calculation of the mean torque over a 500 ms period immediately prior to TMS at the start and end of each sustained fatiguing contraction, during control and recovery MVCs and during the submaximal contractions at 60% and 80% MVC.

Statistical Analysis

Data are reported as means ± SD within the text and displayed as means ± SEM in the figures. For study one, two-way ANOVAs with repeated measures over time and sex

as a between-subject factor (men vs. women) were used to compare the various dependent variables. Statistical design were as follows: (1) fatigue (before vs. after the fatiguing contraction) × sex for comparison of MVC, superimposed twitch, voluntary activation, estimated resting twitch, MEP area, silent period duration and peak relaxation rate of muscle fibers; and (2) time (0, 25, 50, 75, 100% of time to failure) × sex for RMS EMG, MAP, heart rate, RPE and force fluctuations during the fatiguing contraction. A separate repeated measures ANOVA was used to compare baseline measures with those immediately after the fatiguing contraction (time effect at task failure) and an additional repeated measures ANOVA was used to compare recovery measures (time effect during recovery). Group differences in men and women for the time to task failure, various physical characteristics, STAI (trait) levels and physical activity levels were compared using either a parametric or nonparametric test. When normality of distribution within the data can be assumed using the Shapiro-Wilk test, independent t-tests were used to compare differences between men and women for those variables. The Levene's test for equality of variance was used to test for homogeneity of variance between groups. When the normality of distribution within the data could not be assumed, the Mann Whitney U non-parametric test was used to observe differences between men and women. Stepwise linear regression was performed to determine the contribution of dependent variables to the total variation in the time to task failure of men and women (SPSS version 19).

For study two, ANOVAs with repeated measures for task and over time with sex (men and women) as a between-subject factor were used to compare the various dependent variables. Repeated measures factors included session (control and stimulation sessions), stimulation (baseline, after initial stimulation, after fatiguing task

and recovery), time (0, 25, 50, 75, 100% of time to failure) and fatigue (before and after the fatiguing contraction). Specifically, the statistical designs were as follows for the dependent variables: (1) session × sex for time to task failure; (2) session × fatigue × sex for comparison of MVC; session × time × sex for levels of anxiety (VAS) throughout the session and (4) session × time × sex for RMS EMG, MAP, heart rate, RPE and force fluctuations during the fatiguing contraction. Rates of change for several variables were calculated for each subject as the absolute difference from the start to the end of the contraction divided by the time to task failure. The strength of an association is reported as the squared Pearson product-moment correlation coefficient (r^2). A significance level of $P < 0.05$ was used to identify statistical significance.

RESULTS

Study One: Sex Differences and Supraspinal Fatigue

Men and women were similar in age ($P > 0.05$) but differed in height, body mass, physical activity levels and STAI scores ($P < 0.05$, Table 1). Men were twice the strength of women ($P < 0.05$) before and after the fatiguing contraction and their reductions in strength at task failure were similar ($P > 0.05$) (Table 1). Time to task failure however, was longer for women than men ($P = 0.009$, Table 1). MVC torque was negatively associated with time to task failure ($r = -0.45$, $r^2 = 0.20$, $P = 0.01$) indicating that the stronger subjects had a briefer time to task failure. When men and women were analyzed separately the associations were not significant (men: $r = -0.28$, $P = 0.34$; women: $r = -0.19$, $P = 0.49$). Day of menstrual cycle was not associated with time to failure for women ($r = 0.35$, $r^2 = 0.12$, $P = 0.2$), suggesting that hormonal fluctuations did not influence their endurance times.

Variable	Men n = 14	Women n = 15	P value (Sex effect)
Age	20.1 ± 1.9 yr	19.9 ± 3.2 yr	$P = 0.20$
Height	179.7 ± 4.0 cm	167.2 ± 6.7 cm	$P < 0.001$
Body Mass	78.7 ± 9.2 kg	67.6 ± 8.6 kg	$P = 0.002$
STAI (trait)	31.4 ± 7.5	33.9 ± 6.8	$P = 0.35$
PA (METS)	55.5 ± 39.5	46.0 ± 34.8	$P = 0.95$
MVC	81.9 ± 16.0 Nm	40.3 ± 6.2 Nm	$P < 0.001$
Reduction in MVC	41.3 ± 10.0 %	43.1 ± 10.3 %	$P = 0.63$
Time to failure	8.3 ± 2.7 min	12.9 ± 6.3 min	$P = 0.009$

Table 2.1. Subject Characteristics, Strength and Time to Failure for Men and Women in Study 1. The last column indicates the p values for the comparison of men and women. STAI, state trait anxiety inventory; PA, physical activity questionnaire; METS, Metabolic Equivalents· heart rate/week); MVC, maximal voluntary contraction.

Force Fluctuations

Force fluctuations (CV, %) increased throughout the fatiguing contraction (time effect, $P < 0.001$) for both men and women (time × sex interaction, $P = 0.15$, Figure 2.2A) with no main effect of sex, ($P = 0.50$). The rate of increase was more rapid for the men than the women (0.56 ± 0.22 and 0.38 ± 0.21 % $\cdot min^{-1}$, respectively, $P = 0.03$).

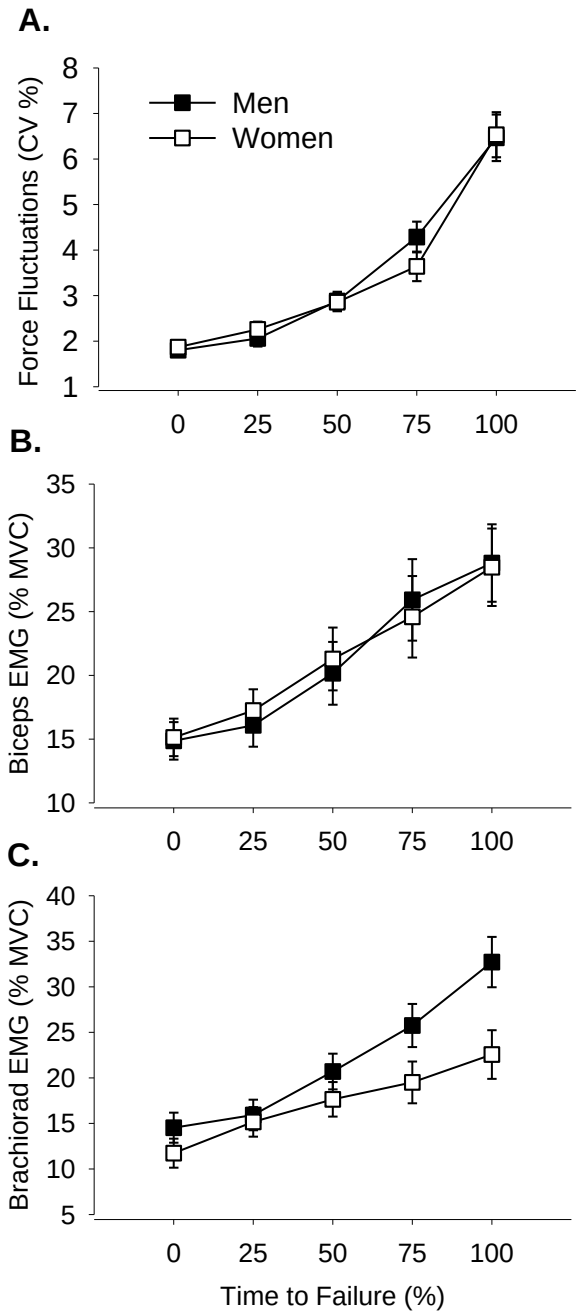

Figure 2.2. Force Fluctuations and EMG Activity during the Fatiguing Contraction. A. Force Fluctuations (CV, %) increase for men (closed squares) and women (open squares). *B-C.* Root mean square (RMS) EMG (% MVC) of the biceps brachii **(*B*)** and brachioradialis **(*C*)** for men (closed squares) and women (open squares) during the fatiguing contraction. Each data point represents means ± SE at 25% increments of the time to task failure for a 30 s interval.

EMG Activity

Biceps brachii EMG activity (% MVC) increased throughout the fatiguing contraction (time effect, $P < 0.001$) similarly for men and women (time × sex interaction, $P = 0.41$, Figure 2.2B) with no main effect of sex ($P = 0.95$). The rate of increase in EMG activity did not differ for men and women (1.7 ± 1.0 and 1.2 ± 1.1 %·min^{-1},

respectively, $P = 0.18$). Brachioradialis EMG activity (% MVC) increased throughout the fatiguing contraction (time effect, $P < 0.001$), however, this increase was different for men and women (time × sex interaction, $P = 0.001$, Figure 2.2C). Men had a greater increase in EMG than the women for the brachioradialis during the fatiguing contraction (2.2 ± 1.2 and 0.8 ± 0.4 %·min^{-1}, respectively, $P = 0.001$). Triceps EMG activity increased throughout the fatiguing contraction for both men and women (time effect, $P = 0.04$) with no interaction of time by sex ($P = 0.61$) and no main effect of sex ($P = 0.47$).

Rating of Perceived Exertion (RPE)

RPE increased for men and women throughout the fatiguing contraction (time effect, $P < 0.001$) with no interaction of time by sex ($P = 0.12$) and no main effect of sex ($P = 0.53$). The rate of rise for RPE was greater for men than for women (0.9 ± 0.1 and 0.6 ± 0.1 RPE·min^{-1} respectively, $P < 0.001$).

Voluntary Activation

The increments in torque generated by stimulation to the motor cortex during the MVCs (SIT) were expressed relative to the torque just prior to the stimulation. SIT increased from baseline to immediately after task failure for both men and women (2.5 ± 2.5 Nm to 7.4 ± 5.0 Nm for men vs. 3.2 ± 2.2 Nm to 9.3 ± 6.6 Nm for women, time effect, $P < 0.001$) with no time by sex interaction ($P = 0.55$). Voluntary activation which was calculated from the SIT, was similar at baseline (during control MVCs) for men and women (93.2 ± 4.8% vs. 92.1 ± 3.9% respectively, sex effect, $P = 0.51$). The reduction in voluntary activation after the fatiguing contraction (time effect at task failure, $P < 0.001$) was similar for the men and women (time × sex interaction, $P = 0.45$, Figure 2.3A).

During recovery, voluntary activation increased similarly for both men and women (time effect during recovery, $P = 0.001$) indicating that they were almost fully recovered (92.0 ± 7.1% for men and 88.9 ± 6.5% for women) after five minutes of recovery (time × sex interaction, $P = 0.34$).

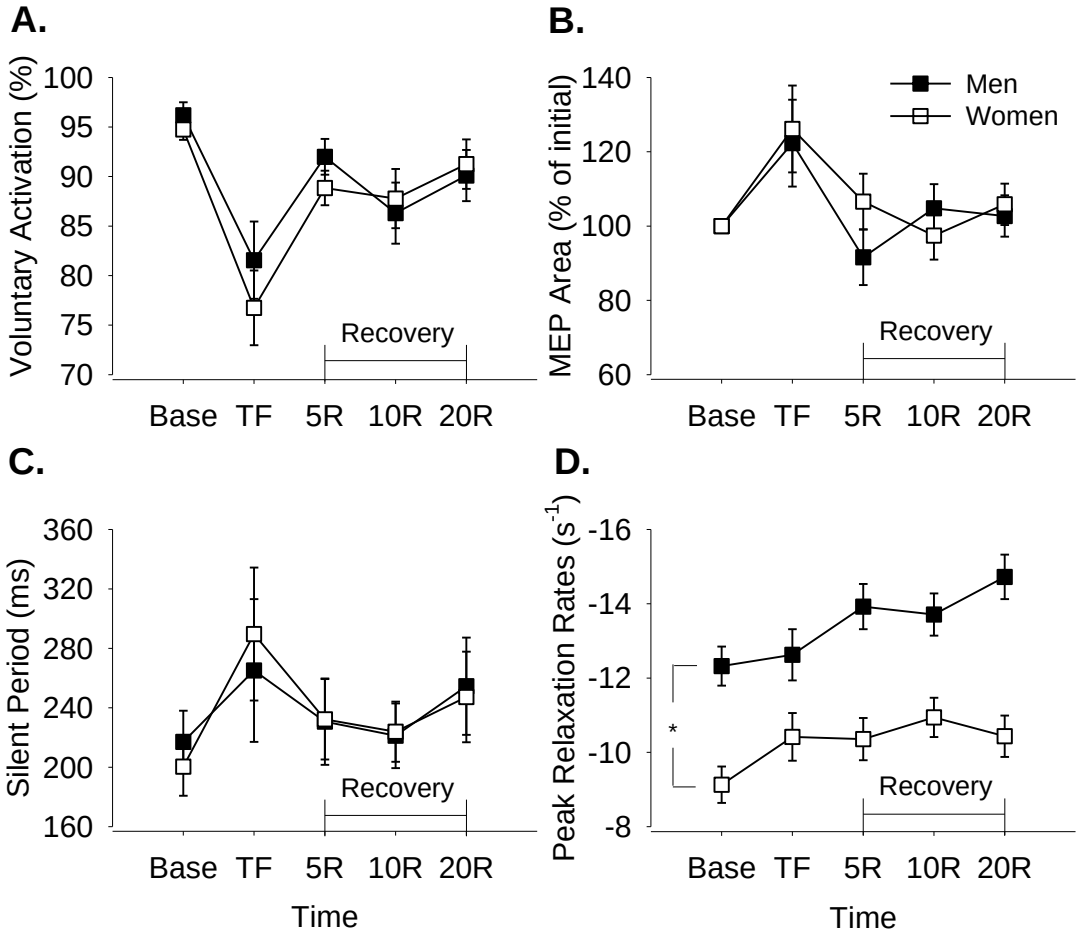

Figure 2.3. Voluntary Activation (*A*), Motor Evoked Potential (MEP) area (*B*), Silent Period (*C*) and Peak Relaxation Rates (*D*) for the biceps brachii muscle. Values are represented as the mean ± SE for men (closed squares) and women (open squares). Base indicates baseline measures before the fatiguing contraction, TF indicates measures recorded immediately upon task failure and 5R, 10R and 20R indicates 5 min, 10 min and 20 min of recovery respectively. * indicates the sex difference in peak relaxation rates, $P < 0.001$.

Motor Evoked Potentials

Biceps brachii, brachioradialis and triceps brachii MEPs were evoked during the MVCs at baseline, immediately upon task failure and during recovery. The M wave area

(measured from brachial plexus stimulation during the MVC) did not change between baseline and after the fatiguing contraction (time effect, $P = 0.48$). MEPs are therefore presented relative to their baseline values. Biceps brachii MEP area increased similarly for both men and women during the MVC from baseline to task failure (time effect at task failure, $P = 0.003$, Figure 2.3B) and returned to baseline for men and women from task failure within 5 min of recovery (time effect after 5 min recovery, $P = 0.001$). Brachioradialis MEP area also increased from baseline to task failure when measured during the MVC for both men and women (time effect, $P = 0.001$) with no time by sex interaction ($P = 0.67$). Triceps MEP area did not change from baseline measures to task failure measured during the MVC (time effect, $P = 0.65$) for men or women (time × sex interaction, $P = 0.18$).

Biceps brachii MEPs were also elicited at the start and end of the fatiguing contraction. MEP area of the bicep brachii increased between the start of the 20% contraction and task failure (time effect, $P = 0.001$) with no interaction of time by sex ($P = 0.21$) and no effect of sex ($P = 0.21$). Neither the M wave area nor amplitude changed from the start of the 20% contraction to task failure ($P > 0.05$).

Silent Period

Silent period evoked during the MVCs increased in duration from baseline to task failure (time effect at task failure, $P = 0.001$) similarly for both men and women (time × sex interaction, $P = 0.36$, Figure 2.3C). The silent period recovered to baseline values within 5 minutes after the fatiguing contraction (time effect during recovery, $P = 0.02$) similarly in men and women (time × sex interaction, $P = 0.99$).

Estimated Resting Twitch

The amplitude of the estimated resting twitch decreased (time effect at task failure, $P < 0.001$) after the fatiguing contraction from baseline values for men (23.4 ± 5.7 to 19.9 ± 5.9 Nm) and women (13.6 ± 2.7 to 11.2 ± 4.1 Nm). The estimated resting twitch amplitude was greater for men than women before and after the fatiguing contraction (sex effect, $P < 0.001$) and there was no interaction of time by sex ($P = 0.43$). The percent decline in the amplitude of estimated resting twitch after the fatiguing contraction was similar for men and women (14.1 ± 16.6% vs. 16.4 ± 27.8%, respectively, $P = 0.79$). Baseline estimated resting twitch values correlated with maximal torque ($r = 0.72$, $r^2 = 0.52$, $P < 0.001$) indicating that the stronger subject had a larger resting twitch amplitude.

Peak Relaxation Rate of Muscle Fibers

Peak relaxation rates (measured during the TMS-induced silent period) were greater for men than women during the MVCs at baseline (-12.3 ± 1.5 s^{-1} vs. -9.1 ± 2.2 s^{-1}, respectively, $P < 0.001$, Figure 2.3D). Peak relaxation rates were not significantly different after the fatiguing contraction compared with before fatigue for both men and women but there was a trend for peak relaxation rates to increase after fatigue (time effect, $P = 0.07$). There was no time by sex interaction ($P = 0.24$), indicating that men and women had similar changes in peak relaxation rates after the fatiguing contraction. During recovery, peak relaxation rates continued to increase for men and women (time effect, $P = 0.02$) but more so for the men than the women (time × sex interaction, $P = 0.01$).

Initial peak relaxation rates were associated with time to task failure (r = 0.37, r^2 = 0.13, P = 0.05) and initial MVCs (r = -0.65, r^2 = 0.42, P < 0.001). Thus, individuals with a briefer time to task failure and stronger muscles had faster peak relaxation rates.

Cardiovascular Measurements during the Fatiguing Contraction

MAP increased during the fatiguing contraction (time effect, P < 0.001, Figure 2.4A). Men had a greater rate of increase in MAP during the fatiguing contraction (P = 0.01). The rate of increase for men was 5.1 ± 2.7 $mmHg \cdot min^{-1}$ and for women was 1.9 ± 1.1 $mmHg \cdot min^{-1}$ throughout the fatiguing contraction (P < 0.001). The increase in MAP was positively associated with MVC torque (r = 0.60, r^2 = 0.36, P = 0.001) indicating that stronger subjects had a greater increase in MAP by the end of the fatiguing contraction.

Heart rate increased during the fatiguing contraction (time effect, P < 0.001, Figure 2.4B). There was no interaction of time by sex (P = 0.2), but overall men had a greater heart rate than women throughout the fatiguing contraction (sex effect, P = 0.01). The rate of increase for men during the fatiguing contraction was 2.4 ± 2.5 $b \cdot min^{-1} \cdot min^{-1}$ and for women 1.1 ± 0.8 $b.min^{-1} \cdot min^{-1}$ (P = 0.07).

Stepwise Regression Analysis

Stepwise linear regression analysis indicated that the primary and only significant predictor for time to task failure of men and women was the baseline maximal voluntary torque (r = 0.453, P = 0.015) which explained 20% of the variance (r^2 = 0.21). Thus, stronger individuals exhibited a briefer time to task failure.

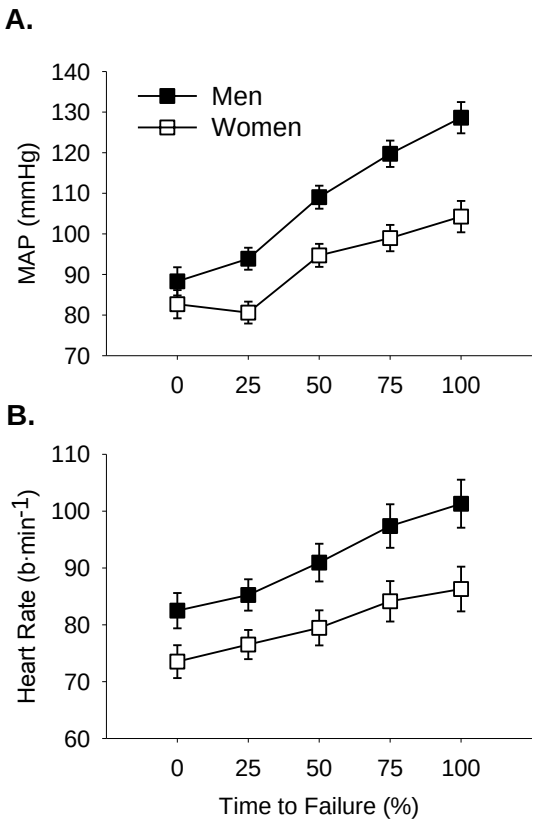

Figure 2.4. Mean Arterial Pressure (MAP; *A*) and Heart Rate (*B*) during the Fatiguing Contractions. The values are presented as mean ± SE at 25% increments of the time to task failure for men (closed squares) and women (open squares). Averages of 15-s intervals were used for the MAP and heart rate.

Study Two: Sex Differences in the Presence and Absence of Stimulation

There was no difference in time to task failure or MVC torque (before and after the fatiguing contraction) in the presence and absence of stimulation ($P > 0.05$, Figures 2.5A and 2.5B) for both men and women. There was no difference in the increase in force fluctuations between the control and stimulation sessions (session effect, $P = 0.71$, Figure 2.5C) for both men and women (session × sex interaction, $P = 0.21$). EMG activity also increased similarly for the biceps brachii and brachioradialis during the fatiguing contraction in the control and stimulation sessions ($P > 0.05$) for both men and women ($P > 0.05$). Furthermore, there was no effect of the stimulation on RPE, heart rate and mean arterial pressure ($P > 0.05$) during the fatiguing contraction for both men and women ($P > 0.05$). Thus, any sex differences in time to failure, MVC force, force

fluctuations, EMG activity, heart rate, mean arterial pressure and RPE was not altered during the stimulation session compared with control.

VAS scores for anxiety and the State STAI anxiety scores were however, slightly elevated throughout the stimulation session compared with the control sessions ($P < 0.05$) but there was no change or increase after the initial exposure to the stimulation prior to the fatiguing contraction ($P > 0.05$).

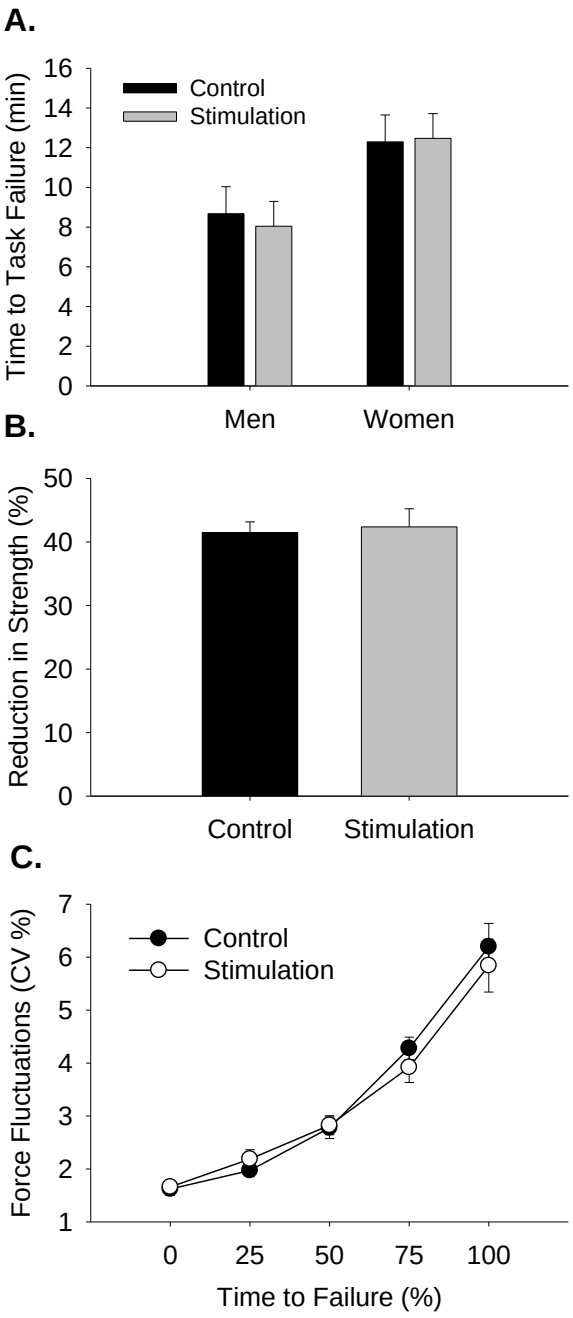

Figure 2.5. Time to Task Failure (*A*), Reduction in Strength (*B*) and Force Fluctuations (*C*) for the Control and Stimulation Sessions. Time to failure was briefer in men compared with women ($P<0.05$) but similar for the control and stimulation session ($P>0.05$) for men and women ($P>0.05$) **(*A*)**. Reduction in strength was similar for the control and stimulation session for men and women (combined) ($P>0.05$) **(*B*)**. Force fluctuations were similar for the control and stimulation session throughout the fatiguing contraction ($P>0.05$) **(*C*)**. Shown is the mean (± SEM) of 15 s intervals in 25% increments of the time to task failure.

DISCUSSION

The novel findings from these studies were that: 1) supraspinal fatigue contributed to neuromuscular fatigue after a low-force isometric fatiguing contraction and this was similar for men and women, 2) the greater fatigue resistance in women was

related to muscular mechanisms that included slower contractile properties and lower absolute strength sustained during the fatiguing contraction and 3) cortical and brachial plexus stimulation did not influence the fatigability or the increase in fluctuations in motor output of the elbow flexor muscles in young men and women, despite some indicators that the men and women were slightly more anxious with the stimulation procedures. Thus, the stimulation used to induce increases in arousal but applied to investigate supraspinal and spinal contributions to muscle fatigue, neither influenced fatigability of the muscle and variability in motor output, nor the sex difference in muscle fatigue.

Supraspinal Fatigue and Sex Differences in Fatigability

Women were less fatigable than men for the low-force isometric contraction (Hunter, 2009). Furthermore, men and women had similar declines in MVC force indicating that both groups had reached similar physiological states at task failure. This was also demonstrated by the similar ratings of perceived exertion at task failure for the men and women. The reduction in MVC force was accompanied by an increase in force evoked by cortical stimulation during the MVC demonstrating that some of the loss in maximal force at task failure was due to suboptimal output from the motor cortex (Gandevia, 2001). There was however, no sex difference in voluntary activation measured during the control MVC before the fatiguing contraction nor immediately after the fatiguing contraction during recovery. Thus, despite the sex difference in performance, both men and women had similar deficits in neural drive to the motor cortex at task failure and after the fatiguing contraction. These results are consistent with other studies for the elbow flexor muscles that demonstrate men and women exhibit

similar magnitudes of supraspinal fatigue during and after repeated maximal contractions (Hunter et al., 2006a) and central fatigue upstream of the motor neuron after a low-force fatiguing contraction (Yoon et al., 2007). These findings for the elbow flexor muscles however, are in contrast to those for leg muscles because reductions in voluntary activation quantified with motor nerve stimulation contributed to a sex difference in knee extensor (Martin & Rattey, 2007) and dorsiflexor muscles (Russ & Kent-Braun, 2003) after repeated maximal contractions. The differential contribution of central fatigue to the sex difference in muscle fatigue across different muscles is not fully understood but may involve sex-specific actions of Groups III and IV afferents onto the motor neuron pool of different muscle groups (Hunter, 2009). Inhibitory feedback from Group III and IV afferents can play a role in spinal or supraspinal fatigue via inhibition of alpha motor neurons (Taylor & Gandevia, 2008) and differs between extensor and flexor muscles (Martin & Rattey, 2007) and probably differs between men and women (Russ & Kent-Braun, 2003; Martin & Rattey, 2007; Hunter, 2009). Taken together, the sex difference in central fatigue appears to be muscle dependent and the sources (supraspinal vs. spinal) contributing to these sex differences may differ.

There was also no sex difference in the EMG responses elicited from cortical stimulation before or after the fatiguing task. MEPs elicited during the sustained 20% fatiguing contraction and the MVCs before and after the fatiguing contraction increased similarly in men and women due to an increase in corticomotor excitability (Taylor & Gandevia, 2008). Thus, men and women demonstrated similar increases in corticomotor excitability immediately after the low-force fatiguing contraction and during recovery. Factors that contribute to changes in the M wave may contribute to changes in the MEP

during maximal fatiguing tasks (Taylor et al., 1999) but there was no change in the M wave from baseline to fatigue and throughout recovery for either men or women. Thus, there was no impairment in transmission of the action potential across the neuromuscular junction for both sexes and the M wave did not contribute to changes in the MEP for the elbow flexor muscles.

The silent period evoked during the MVCs increased similarly for men and women with fatigue. The silent period represents the inhibition within the motor cortex or the period of silence in the EMG following the MEP, this typically lasts more than 200 ms with a high-intensity stimulus (Taylor et al., 1999). Inhibition of descending drive and reduced excitability of the neurons may contribute to the initial part of the silent period. However, the silent period continues beyond the recovery of motoneuronal excitability at ~100 ms (Inghilleri et al., 1993), so that the latter part of the silent period and its end should reflect the intracortical inhibition during maximal voluntary contractions (Gandevia, 2001), although more recent findings indicate that the long-interval inhibition is also due to inhibition in the spinal cord (McNeil et al., 2009). The similar duration of the silent period in men and women after the low-force fatiguing contraction is consistent with prior studies using maximal isometric fatiguing contractions (Hunter et al., 2006a) showing no sex differences in inhibitory processes within the cortex and spinal cord with fatigue.

Peripheral Contributions to Sex Differences after a Low- Intensity Fatiguing Contraction

The longer time to task failure of the women than the men was related to a sex difference in contractile speed of the muscle and differences in absolute strength exerted during the fatiguing task. The primary predictor of the time to failure of the low-force

task however for the men and women was the absolute strength, which confirms findings from our previous studies (Hunter & Enoka, 2001; Hunter *et al.*, 2004a). Peak relaxation rates also correlated with time to failure indicating that subjects with slower muscle fibers were more fatigue resistant. Peak relaxation rates were faster in men than women before and after the fatiguing contraction, suggesting that men had a greater proportion of type II fibers (Jaworowski et al., 2002) and differences in calcium uptake into the sarcoplasmic reticulum which is associated with fiber types (Hunter et al., 1999). These results contribute to a growing body of literature that show women can have slower contractile properties than men and these contractile properties influence their fatigability (Hunter et al., 2006a; Martin & Rattey, 2007). Peak rates of relaxation however, did not change significantly after the fatiguing contraction for either men or women. The slowing of the muscle that can occur during maximal isometric fatiguing protocols (Hunter et al., 2006a; Martin & Rattey, 2007) usually reflects changes in the excitation-contraction coupling (Fitts, 2011). However, contractile properties do not always slow after low-force fatiguing contractions (Kuchinad et al., 2004) and may be influenced by increases in muscle temperature (Todd et al., 2007). Although the contractile speed did not alter with fatigue there was a reduction in the estimated twitch amplitude. These findings paralleled the change in MVC at task failure because men and women had similar relative reductions for the estimated twitch amplitude and MVC after the low-force fatiguing contraction.

During the fatiguing contraction, the men exhibited a greater rate of increase than the women for several variables that are modulated by mechanisms within the muscle, and in particular absolute strength. Mean arterial pressure for example, increased at a

greater rate for the men than the women and this has been shown for several studies during low-force sustained contractions with the elbow flexor muscles (Hunter & Enoka, 2001; Hunter et al., 2004b; Yoon et al., 2007). Presumably, men who are stronger than women, had greater intramuscular pressures, more occluded blood flow, a greater buildup of metabolites, greater activation of metaboreceptors (Group III and IV afferents) and larger rates of increase in mean arterial pressure (pressor reflex) (Rowell & O'Leary, 1990). The pressor response was correlated with the absolute strength, which has been observed previously (Hunter et al., 2004b). MAP is also regulated by central command which is the parallel activation of neural circuits in the brain stem and spinal cord that control motor, ventilatory and cardiovascular function (Hayes et al., 2002). Accordingly, heart rate and RPE which are regulated by central command increased at greater rates for men than women during the fatiguing contraction indicating men required a greater rate of increase in central command to sustain the fatiguing task (Gandevia & Hobbs, 1990). Fluctuations in motor output (force fluctuations) also increased for men at a greater rate than women ending at similar values for both sexes. The increase in fluctuations of motor output during a fatiguing contraction is due to both neural and muscular mechanisms (Cresswell & Loscher, 2000). Muscle fatigue can result in a decrease in the motor unit discharge rate that can increase the discharge rate variability and consequently increase force fluctuations (Mottram et al., 2005). Collectively, these physiological adjustments that are modulated by both central command and muscular processes during the fatiguing contraction reflect an increased rate in the development of muscle fatigue during submaximal tasks in men compared with women.

Men and women also appeared to adopt different activation strategies among the elbow flexor muscles during the fatiguing contraction. In contrast to the biceps EMG, the brachioradialis EMG increased at a much greater rate in the men than the women with the men showing higher activation than the women at task failure. Because the amplitude of the EMG is related to the net motor activity that represents the recruitment and the discharge rates of active motor units (Riley et al., 2008), these results indicate that the rates of increase in recruitment and discharge of motor units of men and women differed among the elbow flexor muscles. The reason for the differences is not clear but may represent physiological sex differences that influence the rate of fatigue in the brachioradialis relative to the biceps brachii. While there are differences in fiber types between the two muscles (Johnson et al., 1973) it is unknown whether there is a sex difference in the fiber type composition within the brachioradialis. Alternatively, the biceps brachii of the men may have been more inhibited than the women via afferent feedback from brachioradialis (Barry et al., 2008), although any sex or strength differences during sustained contractions have not been identified. Ultimately the strategy adopted by the men and women differed in that men required greater activation of the brachioradialis than women to sustain the required force.

Stimulation of the Nervous System Does Not Influence the Sex Difference in Muscle Fatigue

An important finding in this study was that the magnitude of muscle fatigue (reduction in MVC force) at task failure, the time to task failure and the increase in fluctuations of motor output did not change for either men or women when stimulation was applied at the motor cortex and motor nerve in order to quantify supraspinal fatigue

compared with control conditions (no stimulation). Time to task failure was reduced with exposure to acute cognitive stress (Yoon et al., 2009) and steadiness was reduced with exposure to noxious electrical stimulation (Noteboom et al., 2001a; Christou et al., 2004) more so for women than men. However, none of our measures of neuromuscular performance were different in men or women when exposed to the motor cortical and brachial plexus stimulation. Furthermore, average EMG activity of the elbow flexor muscles and RPE were also similar during the two sessions demonstrating similar increases in neural activation (motor unit recruitment and discharge rates) (Riley et al., 2008) and perceived levels of activation. Thus, the current study indicates that the single-pulsed stimuli delivered at high intensities over the motor cortex and motor nerve does not influence time to task failure or other indicators of motor performance during a fatiguing contraction for men and women.

Accordingly, the physiological measures of stress and arousal that included heart rate and mean arterial pressure were similar during the control and stimulation sessions. These are indicators of sympathetic activity which can be elevated with arousal and increased stress (Noteboom et al., 2001a; Yoon et al., 2009) and more so in women than men. Perceived anxiety measures (VAS and STAI state) of the subjects however, were greater during the stimulation session than the control session for both men and women. The levels of increased perceived arousal however were minimal (Christou et al., 2004) and there was no sex difference. Despite the increased levels of anxiety throughout the stimulation session, this did not produce a greater physiological stress response (heart rate and blood pressure) or effect muscle fatigue in either men or women.

In conclusion, supraspinal fatigue contributed to neuromuscular fatigue for a low-force fatiguing contraction but did not explain the sex differences in time to task failure. Rather, this study indicates that the mechanisms contributing to the sex difference in muscle fatigue were attributable to muscular mechanisms that involved a sex difference in absolute muscle strength exerted during the task and initial peak relaxation rates. The stimulation techniques used to quantify supraspinal fatigue did not alter the sex difference in fatigue and the physiological adjustments during a low-force fatiguing contraction. Thus, supraspinal fatigue can be quantified in both men and women without influencing motor performance.

Chapter III

Mechanisms of Increased Muscle Fatigability with Exposure to a Cognitive Stressor

SUMMARY

The purpose of this study was to determine the contribution of contractile properties and supraspinal fatigue to stress-induced changes in muscle fatigability in the upper limb muscles. Twenty-eight young adults (14 women, 14 men, 20 ± 3 years) participated in two experimental sessions (control and stressor). A subset of the subjects participated in a third experimental session (mental attentiveness) (14 men, 9 women). Each session involved an isometric fatiguing contraction (20% of maximal strength) with the elbow flexor muscles. The stressor session included a difficult mental-math task and the mental-attentiveness session a simple mental-math task before and during the fatiguing contraction. Anxiety levels increased after exposure to the stressor ($P < 0.05$). Time to failure was briefer for the stressor session than the control session (9.0 ± 3.3 vs. 10.9 ± 5.2 min, respectively, $P = 0.002$) with a 21.9% reduction for women and 10.6% for men (sex × session, $P = 0.07$). The superimposed twitch from cortical stimulation increased and voluntary activation decreased (95 ± 4 to 79 ± 14%, $P < 0.001$) similarly for men and women across sessions after the fatiguing contraction ($P > 0.05$). Initial peak relaxation rates were more rapid for men than women ($P < 0.05$) but did not differ between sessions after the fatiguing contraction for either sex ($P > 0.05$). Maximal strength however, correlated with the difference in time to failure between the control and stressor session ($r = -0.42$, $r^2 = 0.18$, $P = 0.03$). Time to task failure, voluntary activation

and peak relaxation rates were similar across the control and mental-attentiveness sessions for both men and women ($P > 0.05$). The greater fatigability with a cognitive stressor was not due to differences in supraspinal fatigue or peak relaxation rates of the muscle at task failure but can be explained, in part, by the strength of the individual and possibly stress-induced differences in blood flow regulation of the upper limb.

INTRODUCTION

Psychosocial stress can impair motor performance during low-intensity isometric tasks. When young adults for example, are exposed to an acute stressor (such as difficult mental math or unpredictable delivery of electrical stimulation) while sustaining isometric contractions with the upper limb, muscle fatigue and amplitude of force fluctuations increase (Noteboom et al., 2001b; Christou et al., 2004; Yoon et al., 2009). The influence of stress appears to be more prominent in women than men and in individuals with higher trait anxiety (Noteboom et al., 2001a; Mottram et al., 2006; Yoon et al., 2009). Because physiological responses to psychosocial stress are important determinants of health (Kajantie & Phillips, 2006), the added impact of increased arousal (stress) during low-intensity fatiguing motor tasks that are commonly performed during daily tasks, have important clinical implications in populations vulnerable to motor deficits.

We recently reported that increased fatigability of a submaximal sustained contraction (reduction in time to failure) when performing a difficult mental-math task (cognitive stressor) was in part explained by the initial strength of the individual (Yoon et al., 2009). The impairment in motor performance was greater in weaker subjects who are usually women and was also accompanied by an increase in indices of sympathetic

activation (mean arterial pressure and heart rate) (Yoon et al., 2009). We proposed that the greater fatigue in women when exposed to stress could be due to a selective influence of sympathetic activity on the different fiber type proportions in men and women. Men for example usually possess a greater proportional area of Type II fibers compared with women (Simoneau & Bouchard, 1989; Porter et al., 2002). Increased sympathetic activation can alter the intrinsic properties of muscle fibers by potentiating force of Type II fast-twitch fibers but reducing the force of Type I slow-twitch fibers in animal and human muscle (Bowman, 1980; Roatta et al., 2008). An aim of this study was to determine the association between contractile properties that can reflect sex differences in fiber types and the change in fatigue when a cognitive stressor is imposed.

Sex differences in the stress response however are also centrally mediated (Valentino & Van Bockstaele, 2008). Activation of the sympathetic nervous system that occurs with stress causes release of neuromodulators (norepinephrine and serotonin) and hormones (epinephrine and cortisol) that impact cognitive (Lupien et al., 2007), cardiovascular (Herd, 1991) and motor functions (Lupien et al., 2007; Valentino & Van Bockstaele, 2008). Sex-specific hormones, i.e. estrogen, influence the synthesis of neuromodulators and stress hormones resulting in sex differences in cognitive and motor processing (Halari et al., 2005). There are also sex differences in brain activation patterns during stressful cognitive tasks (Wang et al., 2007) and motor tasks (Wong et al., 2007) and descending command and synaptic input to the motoneuron pool is altered during tasks that require more attention (Johansen-Berg & Matthews, 2002). Stress-induced alterations in muscle fatigability therefore could involve adjustments from supraspinal sources that differ between men and women.

Under control conditions women are able to sustain fatiguing contractions longer than men (Hunter, 2009) but the magnitude of central fatigue and supraspinal fatigue is similar for both sexes (Chapter II & Hunter et al., 2006a). Central fatigue is an exercise-induced reduction in neural drive to the muscle (voluntary activation) and supraspinal fatigue is a component of central fatigue which is attributable to suboptimal output from the motor cortex (Gandevia, 2001). Supraspinal fatigue is measured as an exercise-related decline in voluntary activation with cortical stimulation (Gandevia, 2001; Taylor & Gandevia, 2008). It is unknown whether the greater fatigue associated with stress in women can be attributed to supraspinal fatigue.

The purpose of this study therefore was to determine the contribution of contractile properties and supraspinal fatigue to stress-induced changes in muscle fatigability in the upper limb muscles. We hypothesized that weaker adults (usually women) would have greater decrements in time to failure of a submaximal isometric fatiguing contraction when exposed to a cognitive stressor and this would be associated with slower rates of relaxation and greater supraspinal fatigue in the elbow flexor muscles. Transcranial magnetic stimulation (TMS) was used to quantify supraspinal fatigue and peak relaxation rates before and after the low-intensity fatiguing contraction of the elbow flexor muscles.

METHODS

Twenty-eight young adults (14 women) participated in a familiarization session and two experimental sessions (control and stressor sessions). A subset of the subjects participated in a third experimental session (mental attentiveness) (14 men, 20 ± 2 years and 9 women, 20 ± 4 years). The order of the sessions was counterbalanced among the

subjects. Experimental sessions were ≥7 days apart and involved performing a fatiguing contraction with the elbow flexor muscles of the left arm. Prior to participation in the study, each subject provided informed consent. The protocol was approved by the institutional review board at Marquette University. All subjects were healthy with no known neurological or cardiovascular diseases and were naive to the protocol. Subjects reported no history or current mental pathology, including anxiety disorders and/or depression. Trait (general) levels of anxiety were quantified by the State Trait Anxiety Inventory (STAI) (Spielberger, 1970).

At the familiarization session, each subject practiced maximal voluntary contractions (MVC) with the elbow flexor muscles and was habituated to the stimulation procedures that included TMS and electrical stimulation of the brachial plexus. The physical activity level for each subject was assessed with a questionnaire that estimated the relative kilocalorie expenditure per week (Kriska et al., 1990). Hand dominance was estimated by the Edinburgh Handedness Inventory (Oldfield, 1971) [0.53 ± 0.3 vs. 0.63 ± 0.2 for men and women, respectively, with a ratio of 1 indicating complete right-handedness]. For each female participant, the day of their menstrual cycle was recorded for each experimental day. The first day of menstruation was considered day one of the cycle.

Experimental Sessions Overview

In the control session, each subject performed the contraction without performing the cognitive tasks (i.e. mental-attentiveness or mental-math tasks). During the cognitive sessions, each subject was required to perform 4 minutes of a mental-math or a mental-attentiveness task before and simultaneously while performing the fatiguing contraction

(see *Experimental Protocol* for more detail). Heart rate and mean arterial pressure (MAP) were measured during the mental-math and mental-attentiveness tasks at rest and during contraction. Anxiety using the visual analogue scale (VAS) and STAI (state) were measured before and after performance of the cognitive tasks (see Figure 3.1). The fatiguing contraction involved maintaining a target level that was equivalent to 20% of MVC force for as long as possible. TMS (elicited during MVCs and submaximal contractions) was used to quantify voluntary activation as a measure of supraspinal fatigue, motor evoked potentials (MEP), silent period duration and peak relaxation rates before and after the fatiguing contraction.

Mechanical Recordings

Each subject was seated upright in an adjustable chair with the left arm slightly abducted and the elbow joint resting on a padded support. The elbow joint was flexed to 90 degrees so that the forearm was parallel to the ground and the force at the wrist was directed upward with activation of the elbow flexor muscles. The hand and forearm were placed in a modified wrist-hand-thumb orthosis (Orthomerica, Newport Beach, CA) and the forearm was placed midway between pronation and supination. Two nylon straps were placed over each shoulder to minimize shoulder movement. The force exerted by the wrist in the vertical direction was measured with a transducer (JR-3 Force-Moment Sensor; JR-3 Inc., Woodland, CA) that was mounted on a custom-designed, adjustable support. The orthosis was attached to the force transducer. The force was recorded on-line at 500 samples$\cdot s^{-1}$using a Power 1401 A-D converter and Spike 2 software (Cambridge Electronic Design, Cambridge, UK) and displayed on a 19-in monitor 1.5 m in front of the subject. Each subject was asked to trace the horizontal cursor with the

force signal for as long as possible during the fatiguing contraction. The force signal appeared on the screen from the right side of the monitor at 2.5 $cm \cdot s^{-1}$.

Electrical Recordings

EMG signals were recorded with bipolar surface electrodes (Ag-AgCl, 8-mm diameter; 16 mm between electrodes) that were placed over the long head of the biceps brachii, brachioradialis, and long head of the triceps brachii muscles. The bipolar electrode configuration was placed longitudinally over the muscle belly midway between the origin and insertion for each muscle, according to the European recommendations for surface EMG (Hermens et al., 2000). Reference electrodes were placed on a bony prominence at the elbow. The EMG signal was amplified (100×) and band-pass filtered (13-1000 Hz) with Coulbourn modules (Coulbourn Instruments, Allentown, PA) prior to being recorded directly to a computer with the Power 1401 A-D converter and Spike 2 (CED). The EMG signals were digitized at 2000 $samples \cdot s^{-1}$.

Stimulation

Subjects were stimulated at the brachial plexus with electrical stimulation and at the motor cortex with TMS.

Brachial Plexus Stimulation. The brachial plexus was electrically stimulated to produce a maximal compound muscle action potential (maximum M wave: M_{max}) of the biceps brachii, brachioradialis and triceps brachii muscles. A cathode was placed in the supraclavicular fossa and an anode on the acromion process. A constant-current stimulator (model DS7AH, Digitimer, Welwyn Garden City, Hertforshire, UK) was used to deliver single stimuli (100 μs duration) to the brachial plexus.

Motor Cortex Stimulation. TMS was delivered via a round coil (13.5-cm outside diameter) over the vertex (Magstim 200, Magstim, Whitland, UK) to elicit MEPs in biceps brachii, brachioradialis and triceps brachii muscles. The right cerebral hemisphere was stimulated by the direction of the current flow in the coil to preferentially activate the left limb. A single pulse was delivered over the motor cortex at an intensity that produced a large MEP in the agonist biceps muscle (minimum amplitude of 50 % of M_{max} during a brief MVC of the elbow flexor muscles) but only a small MEP in the antagonist triceps muscle amplitude (< 20 % of M_{max}) when the M wave was maximal (Todd et al., 2004). TMS was delivered during voluntary contractions only, including during brief maximal and submaximal contractions prior to and after the fatiguing contraction (recovery), and also during the fatiguing contraction (once at the start and once at task failure) (Figure 3.1).

Cardiovascular Measurements

Heart rate and blood pressure were monitored at rest (baseline), during the cognitive tasks or quiet sitting (prior to the fatiguing contraction) and also during each fatiguing contraction. Both heart rate and blood pressure were monitored with an automated beat-by-beat, blood pressure monitor (Finapres 2300; Ohmeda, Louisville, CO). The blood pressure cuff was placed around the middle finger of the relaxed, right hand with the arm placed on a table adjacent to the subject at heart level. The automated blood pressure signal was calibrated to a manual blood pressure reading for each participant. The blood pressure signal was recorded on-line to a computer at 500 samples$\cdot s^{-1}$.

Cognitive Assessment of Arousal

Cognitive levels of anxiety were assessed throughout the protocol using VAS (Johnson, 2001) and the state portion of the STAI questionnaire (Spielberger, 1970) as we have detailed previously (Chapter II & Yoon et al., 2009). In brief, the VAS involved a 10-cm line anchored at the far left by "not at all anxious" and at the far right by "very anxious." Anxiety was defined as the emotional changes perceived by the subject which was above and beyond the expectation for their level of exertion (Christou et al., 2004). The subject indicated their level of anxiety on the horizontal line of the scale. VAS for anxiety was recorded at 8 time points during the protocol (Figure 3.1): two baseline assessments before intended arousal [T1-T2]; after the first 2-min bout of the cognitive task with no contraction (mental-attentiveness or mental-math session) or quiet rest (control session) [T3]; after the second 2-min bout of the cognitive task or quiet rest (control session) [T4]; immediately after the fatiguing contraction [T5]; and then 5, 10 and 20 minutes after the fatiguing contraction [T6-T8] (recovery).

The STAI-state questionnaire involved 20 statements that required a response on a four-point, Likert-type scale. Assessment of STAI was performed at baseline and after quiet sitting (control session) and after 4 min (2 x 2-min bouts) of the mental-math or mental-attentiveness tasks (cognitive sessions) (Figure 3.1).

Cognitive tasks

Mental math is a well-established psychosocial technique to induce stress (Noteboom et al., 2001b; Kajantie & Phillips, 2006) used in a previous study to increase levels of anxiety and stress (Yoon et al., 2009). During the stressor session, each subject performed serial subtraction from a 4-digit number by 13 with a response required every 3 s (Noteboom et al., 2001b). Once the subject made an error in the math or was not able

to provide the correct answer within 3 s, they were asked to start the mental math again from a new number in the series. Each subject performed the mental math during the stressor session only. They performed the mental math before the fatiguing contraction (2 x 2 min bouts) and then continuously during the fatiguing contraction.

The mental-attentiveness task required subjects to perform a simple math task that was not designed to induce stress (Yoon et al., 2009). Participants subtracted by one from 50 continuously during the 4 minutes (2 x 2 min bouts) prior to the fatiguing contraction and during the fatiguing contraction in the mental-attentiveness session.

Experimental Protocol at Experimental Sessions

Each experimental session began with assessments of baseline levels of anxiety using the VAS and STAI (state levels). After the initial set-up of the subject, stimulation of the motor cortex with TMS and brachial plexus with electrical stimulation was conducted to determine supramaximal levels of stimulation. These levels of stimulation remained constant throughout the rest of the protocol for that session. All procedures thereafter were performed in the following order for each experimental session (Figure 3.1): (1) MVCs of the elbow extensor and elbow flexor muscles, (2) assessment of cognitive and physiological arousal before and after either quiet sitting (control session) or 4 minutes (2 x 2-min bouts) of mental tasks (stressor and mental-attentiveness sessions only), (3) performance of a fatiguing contraction at 20% MVC force, and (4) recovery MVCs and assessment of cognitive and physiological arousal immediately after the fatiguing contraction and at 5, 10 and 20 min recovery.

Pre-fatigue measures. Two MVC*s* of the elbow extensors were performed so that peak EMG values could be obtained to normalize the triceps EMG activity during the

fatiguing contractions. One initial elbow flexor MVC was performed to determine if the subject was within 5% of previous sessions and to calculate force for the submaximal fatiguing contraction at 20% MVC. To quantify voluntary activation, four sets of brief control contractions (2-3 s) with the elbow flexor muscles were separated by 2 minutes of rest to minimize fatigue. Each set involved performance of a MVC followed by brief contractions of 60% and 80% MVC. Within each set of contractions, the start of each contraction was separated by 3-4 s. Peak forces in the MVC from two of the four trials needed to be within 5% of each other, if this did not occur additional trials were performed until this was accomplished. TMS was delivered during each contraction and brachial plexus stimulation was only delivered during the MVCs.

Fatiguing contraction. A fatiguing contraction was performed with the elbow flexor muscles at 20% MVC force during each experimental session. The subject matched the target force as displayed on the monitor and was verbally encouraged to sustain the force for as long as possible. The fatiguing contraction was terminated when the target force declined by 10% of its value. To minimize the influence of transient fluctuations in motor output on the criteria for task failure, the task was terminated only after force fell below the predetermined threshold for 2 out of a 4-s interval. Task failure was detected automatically using a custom-designed program (Spike 2, CED) that monitored the force signal and this time was recorded as the time to task failure.

An index of perceived effort, the rating of perceived exertion (RPE), was assessed with the modified Borg 10-point scale (Borg, 1982; Yoon et al., 2009). Each subject was instructed to focus the assessment of effort on the arm muscles performing the fatiguing task. The scale was anchored so that 0 represented the resting state and 10 corresponded

to the strongest contraction that the upper limb muscles could perform. The RPE was recorded at the beginning of the fatiguing contraction and every minute thereafter until task failure.

Recovery. Measures of MVC force, voluntary activation and VAS for anxiety were assessed at the following times: immediately upon task failure, 5, 10 and 20 min after termination of the fatiguing contraction [T5-T8] (see Figure 3.1).

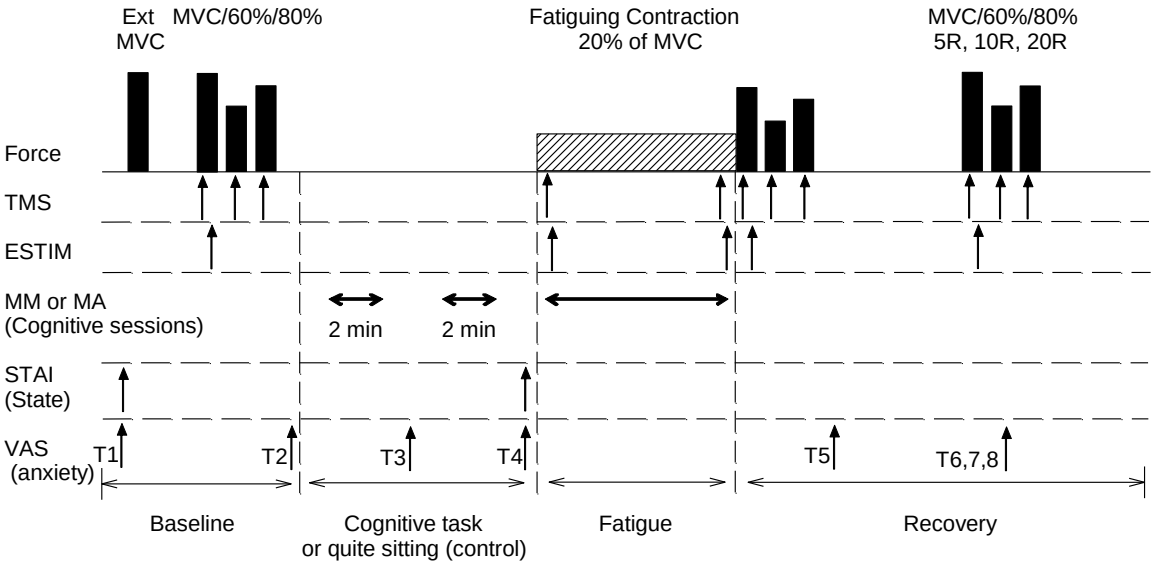

Figure 3.1. Experimental Protocol. The top panel shows the order of force tasks performed by each subject with the elbow flexor muscles or elbow extensors muscles (Ext). Two MVCs with the elbow extensors were performed. This was followed by four MVCs of the elbow flexors and brief contractions at 60 and 80% of MVC which were repeated during recovery from the fatiguing contraction. In the second and third rows, the arrows denote the time points that transcranial magnetic stimulation (TMS) and electrical stimulation of the brachial plexus (ESTIM) were delivered respectively. Mental math (MM), mental attentiveness (MA) or quiet rest (control session) were performed 2 x 2 min (total of 4 minutes) before and then during the fatiguing contraction for each respective session. State-Trait Anxiety Inventory (STAI) questionnaire was assessed twice throughout the protocol. Levels of anxiety using the visual analogue scale (VAS) were assessed throughout the protocol. The bottom panel shows the order in which the events took place (5R, 10R and 20R denotes recovery at 5, 10 and 20 minutes respectively). Note that the schematic is not to scale for time or force.

Data Analysis

The MVC force was quantified as the average value over a 0.5-s interval that was centered about the peak of the MVC. The torque for the MVC and submaximal contractions was calculated as the product of force and the distance between the elbow joint and the point at which the wrist was attached to the force transducer (moment arm).

During the cognitive tasks or quiet sitting, heart rate and MAP were analyzed at the start of the first 2-min bout, and then at 30 s and 90 s after the start of each 2-min bout of the mental tasks. During the mental-attentiveness task, heart rate and MAP (prior to the fatiguing contraction) were unable to be analyzed for 6 subjects (3 men) due to artifact in the signal. This data is therefore not reported. Heart rate and MAP were also recorded during each fatiguing contraction and analyzed by comparing ~15-s averages at 25% intervals. For each interval, the blood pressure signal was analyzed for the mean peaks [systolic blood pressure (SBP)], mean troughs [diastolic blood pressure (DBP)], and number of pulses per second (multiplied by 60 to determine heart rate). MAP was calculated for each epoch with the following equation: MAP = DBP +1/3(SBP – DBP). Rate pressure product was calculated as the heart rate multiplied by the MAP (heart rate × MAP). Rate pressure product is an indicator of cardiac workload and the oxygen requirement of the heart (Wasmund et al., 2002).

The amplitude of the superimposed twitch (SIT) elicited by TMS is reported as a percentage of the voluntary force measured immediately prior to TMS (Gandevia, 2001). The SIT amplitude was also used to calculate voluntary activation. Voluntary activation

was quantified by expressing the amplitude of the SIT (elicited by TMS) as a fraction of the estimated amplitude of the response evoked by the same stimulus at rest (estimated resting twitch, eRT) (Todd et al., 2003). Because motor cortical and spinal cord excitability increase with activity (Hess et al., 1986), a control resting twitch was not able to be achieved at rest. Therefore, the amplitude of the resting twitch was estimated rather than measured directly (Todd et al., 2003). During the sets of brief maximal and submaximal contractions (MVC followed by 60% and 80% MVC contractions), TMS was delivered and the resting twitch was estimated by extrapolation of the linear relation between the amplitude of the SIT and voluntary force. One regression analysis was performed for each set of brief contractions. The y-intercept was taken as the estimated amplitude of the resting twitch evoked by TMS. The amplitude of the estimated resting twitch can be accurately determined from three data points in fresh or fatigued muscle when the contractions are greater than 50% MVC (Todd et al., 2003). Voluntary activation (%) was calculated as a percentage measured by cortical stimulation [(1-SIT/eRT) x 100] (Todd et al., 2003). Data points were excluded (8.6%) for subjects at different time points when the regression of the estimated twitch was $r < 0.9$.

Contractile properties of the elbow flexor muscle fibers were also assessed. The amplitude of the estimated resting twitch was used as an index of the force generating capacity of the elbow flexor muscles and the decline of the force after cortical stimulus was used to determine the peak relaxation rate of the whole muscle (Todd et al., 2007). Peak relaxation rates were determined during each MVC by calculating the steepest falling of the force during the EMG silence immediately following TMS (Todd et al., 2007). This was determined as the highest negative derivative of the force for an interval

of 10 ms between two cursors placed either side of the decline in force during the silent period. The steepest rate of force decline was normalized to the total force (MVC plus superimposed twitch) prior to the silent period (Todd et al., 2007).

The amplitude and area of MEPs and M wave were measured between two cursors placed at the start and end of the waveform for the bicep brachii, brachioradialis and tricep brachii muscles. Because the M wave amplitude and area showed similar changes throughout, only M wave area is reported. Additionally, the MEP areas for the biceps and brachioradialis muscles were similar in their changes between the stressor and control sessions so only biceps MEP areas are reported. M waves were elicited after each MEP, but because there were no changes in the M wave, the MEP is represented as a percentage change from their baseline values. When TMS is delivered during a voluntary contraction, the MEP is followed by a period of near-silence in the EMG (Inghilleri et al., 1993). The silent period was measured as the interval from the stimulus to the resumption of continuous EMG. Voluntary torque was quantified by calculation of the mean torque over a 500 ms period immediately prior to TMS at the start and end of each sustained fatiguing contraction, during control and recovery MVCs and during the submaximal contractions at 60% and 80% MVC.

Statistical Analysis

Data are reported as means ± SD within the text and table and displayed as means ± SEM in the figures. Separate repeated measure ANOVAs for time and session with sex as a between-subject factor (men vs. women), were used to compare various dependent variables. Repeated measures factors included session (control vs. stressor or control vs. mental attentiveness), fatigue (baseline, after fatiguing task and recovery), time [before

vs. after the cognitive tasks (prior to the fatiguing contraction) and during the fatiguing contraction] (0, 25, 50, 75, and 100% of time to failure). Separate ANOVAs were performed to compare control vs. the stressor session and control vs. mental-attentiveness session because not all subjects completed the mental-attentiveness session. Specifically, the statistical designs were as follows for the dependent variables: (1) session × sex for time to task failure; (2) session × fatigue × sex for comparison of MVC, SIT, voluntary activation, estimated resting twitch, MEP area, silent period duration and peak rates of relaxation; (3), session × time × sex for anxiety, heart rate and MAP before and after the cognitive tasks (prior to the fatiguing contraction); and (4) session × time × sex for MEP area, MAP, heart rate, and RPE during the fatiguing contraction.

Group differences in men and women for various physical characteristics, STAI (trait) levels and physical activity levels were compared using either a parametric or nonparametric test. When normality of distribution within the data can be assumed using the Shapiro-Wilk test, independent t-tests were used to compare differences between men and women for those variables. The Levene's test for equality of variance was used to test for homogeneity of variance between groups. When the normality of distribution within the data could not be assumed, the Mann Whitney U non-parametric test was used to observe differences between men and women. The strength of an association is reported as the squared Pearson product-moment correlation coefficient (r^2). A significance level of $P < 0.05$ was used to identify statistical significance. SPSS version 19 was used for statistical analysis.

Because both men and women demonstrated a reduction in time to task failure during the stressor session, a cluster analysis was performed to determine two groups of

subjects: one group that was not affected by the cognitive stressor (nonresponders) and the other that showed declines in time to failure when exposed to the cognitive stressor compared with their control session (responders). Because the results of the responders vs. the nonresponders were similar to the results for the men vs. women, we have presented the sex differences (men vs. women) results only.

RESULTS

Men and women were similar in age, physical activity and trait anxiety levels ($P < 0.01$), but differed in height and weight ($P > 0.01$, Table 3.1).

Variable	Men n=14	Women n=14	P value (Sex effect)
Age (years)	20 ± 2	20 ± 3	$P = 0.20$
Height (cm)	179.7 ± 4.0	168.6 ± 8.0	$P < 0.001$
Body Mass (kg)	78.7 ± 9.2	68.5 ± 8.1	$P = 0.006$
STAI (trait)	31.4 ± 7.5	33.3 ± 6.7	$P = 0.48$
PA (METS)	55.5 ± 39.5	56.8 ± 46.7	$P = 0.94$

Table 3.1. Subject Characteristics for Men and Women. The last column indicates the p values for the comparison of men and women. STAI, State trait anxiety inventory; PA, physical activity questionnaire; METS, Metabolic Equivalents• heart rate/week

Psychological Levels of Stress

STAI (state) increased from baseline to after the cognitive stressor or quiet sitting (control) more for the stressor session (30.3 ± 7.3 to 39.6 ± 10.2) than the control session (33.2 ± 10.4 to 37.0 ± 11.2, session × time, $P = 0.04$). STAI was similar for both men and women (sex effect, $P = 0.53$) and with no sex differences across time (time × sex, $P = 0.69$). STAI increased from baseline to after the mental-attentiveness task and quiet

sitting (control session) (time effect, $P = 0.006$) similarly (session × time, $P = 0.43$). There was no sex difference ($P = 0.66$) and no interactions ($P > 0.05$).

Perceived anxiety was also measured with the VAS throughout the session. Anxiety levels were greater for women than men at baseline for the stressor (1.5 ± 1.4 vs. 0.64 ± 0.35, respectively, $P = 0.04$) and mental-attentiveness sessions (1.4 ± 1.0 vs. 0.54 ± 0.38, respectively, $P = 0.01$) but was similar for the control session (1.5 ±1.3 vs. 0.94 ± 0.65, respectively, $P = 0.14$). Perceived anxiety increased after the mental-math task during the stressor session (session × time after stress, $P < 0.001$) compared with quiet sitting during the control session. There was a main effect of sex ($P = 0.03$) because women had elevated anxiety at baseline and after stress (or quiet sitting) for both sessions. Hence from baseline, men had a 40% increase and women had a 28% increase in anxiety after exposure to the cognitive stressor. In contrast, anxiety decreased after the mental-attentiveness task more so than for the quiet sitting (control session) (session × time after mental-attentiveness task, $P = 0.002$) for both men and women (session × sex after mental-attentiveness task, $P = 0.41$). There was an overall effect of sex ($P = 0.03$) because women had greater anxiety for both the control and mental-attentiveness sessions compared with men.

Anxiety (VAS) increased when assessed immediately after the fatiguing contraction but more so for the stressor session (45%) compared with the control session (22%) (session × fatigue, $P = 0.03$) for both men and women (sex × session × fatigue, $P = 0.23$). Anxiety (VAS) returned to baseline levels during recovery (time effect during recovery, $P < 0.001$) similarly for the men and women (sex effect, $P > 0.05$). Anxiety

(VAS) also increased after the fatiguing contraction (fatigue effect, $P = 0.005$) similarly for the control and mental-attentiveness sessions (session × fatigue, $P = 0.19$).

Time to Task Failure

Time to failure was briefer for the stressor session compared with the control session (9.0 ± 3.3 vs. 10.9 ± 5.2 minutes respectively, session effect, $P = 0.002$) with an interaction of session and sex ($P = 0.07$, Figure 3.2). The relative reduction in time to failure between sessions was 17.8% (21.9% for women and 10.6% for men). Men had a briefer time to failure than women for both sessions (7.8 ± 2.2 min vs. 12.6 ± 5.1 min respectively, sex effect, $P = 0.004$). Time to task failure however, was similar for the mental-attentiveness and control sessions (session effect, $P = 0.32$, Figure 3.2) for both men and women (session × sex, $P = 0.63$). Neither time to task failure (all three sessions) nor the reduction in time to failure between the control and stressor session were associated with day of the menstrual cycle in the women ($P > 0.05$).

Figure 3.2. Time to Failure. Time to task failure for the control, mental-attentiveness (MA) and stressor sessions for men and women during the 20% fatiguing contraction with the elbow flexor muscles. Men fatigued more quickly than women for all three sessions ($P < 0.01$). Men and women were more fatigable during the stressor session compared with the control session ($P = 0.002$).

Maximal Voluntary Contractions

Men were stronger than women for all three sessions (sex effect, $P < 0.001$). MVC torque was not different between the control and stressor sessions (session effect, $P = 0.70$) or between control and mental-attentiveness sessions (session effect, $P = 0.64$) for men or women for each analyses (session × sex, $P > 0.05$, Figure 3.3). Additionally, reductions in MVC torque were similar between sessions for both men and women. Initial MVC torque however, was negatively correlated with the relative and absolute difference in time to task failure between the control and stressor session (relative difference: $r = -0.39$, $r^2 = 0.15$, $P = 0.04$, Figure 3.7 and absolute difference: $r = -0.42$, $r^2 = 0.18$, $P = 0.03$). Thus, weaker individuals had a greater decrement in the time to failure in the stressor session compared with control. When the correlations were done separately for men and women, they were not significant (women: $r = 0.08$, $P = 0.70$ and men: $r = -0.31$, $P = 0.15$).

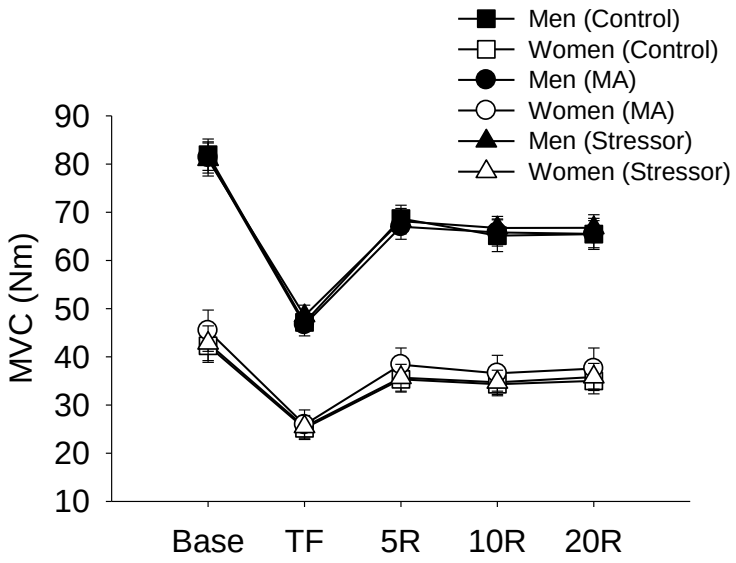

Figure 3.3. Maximal Voluntary Contractions, MVCs. MVC torque for the control, mental-attentiveness (MA) and stressor sessions**.** MVC torque of the elbow flexor muscles for men and women are shown at baseline (Base), at task failure (TF), and 5, 10 and 20 min throughout recovery (5R, 10R, 20R). Values are represented as the mean ± SE for men (closed symbols) and women (open symbols). MVC torque was greater for men than women before, immediately after and throughout recovery for all three sessions ($P < 0.05$). Reduction in strength was similar for men and women across sessions ($P > 0.05$).

Supraspinal Fatigue

Superimposed twitch (SIT). SIT force elicited during MVCs was similar at baseline for the stressor and the control session (session effect at baseline, $P = 0.99$) for both men and women (session × sex, $P = 0.89$). There was no main effect of sex ($P = 0.59$). SIT increased after the fatiguing contraction (time effect, $P < 0.001$) for men and women similarly for both sessions (session × time, $P = 0.93$, Figure 3.4A). There was no effect of sex and no interactions ($P > 0.05$). SIT decreased during recovery (fatigue effect during recovery, $P < 0.001$) but did not fully recover to baseline values by 20 minutes post fatigue for men and women. The time course of recovery was similar for the control and stressor sessions (session × fatigue during recovery, $P = 0.92$) and for men and women (session × fatigue × sex during recovery, $P = 0.27$). Likewise, there were no significant differences between the control and mental-attentiveness sessions in the SIT for men and women ($P > 0.05$).

Voluntary activation. Voluntary activation calculated from the SIT and the estimated resting twitch was similar at baseline for the stressor and the control sessions (session effect at baseline, $P = 0.48$). Reduction in voluntary activation was similar for both sessions (session × fatigue, $P = 0.54$, Figure 3.4B) for men and women (session × fatigue × sex, $P = 0.46$) after the fatiguing contraction. Consequently, the difference in time to failure with exposure to stress was not associated with the difference in voluntary activation between sessions ($P = 0.71$). Voluntary activation increased by 5 minutes of recovery, but was not fully recovered to baseline values within the 20 minutes of recovery period (fatigue effect during recovery, $P < 0.001$). Recovery was similar for the control and stressor sessions for men and women (sex effect, $P = 0.42$) and there were no

interactions of time, session and sex ($P > 0.05$). Voluntary activation was similar for the men and women during the control and mental-attentiveness task from baseline to task failure and throughout recovery ($P > 0.05$).

Motor Evoked Potentials

M wave area of the biceps brachii elicited during MVCs did not change from baseline values to after the fatiguing contraction (fatigue effect, $P = 0.33$) or during recovery (fatigue effect during recovery, $P = 0.44$). M wave area was similar across all three sessions ($P > 0.05$). Hence, any change in MEP size was not due to changes in the size of the M wave.

MEP area of biceps brachii elicited during MVCs increased (fatigue effect, $P < 0.001$) similarly in the stressor and control sessions from baseline to after the fatiguing contraction (126 ± 44 % for the control session and 131 ± 44 % for the stressor session, session × fatigue, $P = 0.68$) for both men and women (session × fatigue × sex, $P = 0.15$). There was no main effect of sex ($P = 0.19$). The MEP area had returned to levels similar to baseline within 5 minutes after the fatiguing contraction (time effect from baseline to 5 min recovery, $P = 0.67$) for both sessions with no interactions ($P > 0.05$). Similar to the control and stressor session, the biceps brachii MEP area during the mental-attentiveness session increased from baseline to after fatigue (fatigue effect, $P < 0.001$), for both men and women ($P > 0.05$).

MEPs during 20% contraction. M wave area did not change from the start of the contraction to just prior to task failure (fatigue effect, $P = 0.87$) for the biceps brachii for all three sessions (fatigue × session, $P > 0.05$). There was no difference in M wave area between men and women ($P > 0.05$).

Biceps brachii MEP area increased during the 20% fatiguing contraction from the start of the task to task failure (time effect, $P < 0.001$) for the control and stressor sessions (session × fatigue, $P = 0.53$). Men and women had similar increases in MEP area (session × fatigue × sex, $P = 0.13$). Biceps brachii MEP area increased from the start of the contraction to task failure (fatigue effect, $P < 0.001$) for the control and mental-attentiveness sessions with no effect of session or sex and no interactions ($P > 0.05$).

Silent Period

Silent period during MVCs. The silent period duration elicited during the MVCs at baseline was longer in the stressor and mental-attentiveness session compared with the control session (228 ± 106 vs. 228 ± 80 vs. 194 ± 69 ms respectively, $P < 0.05$). The silent period increased from baseline to task failure (time effect after fatigue, $P = 0.001$) more so during the control session compared with the stressor session (session × fatigue, $P = 0.02$) and the mental-attentiveness session (session × fatigue, $P = 0.03$) because of the elevated baselines during the cognitive tasks. Therefore, immediately after task failure the silent period was similar for the control (241 ± 21 ms), stressor (234 ± 20 ms), and mental-attentiveness sessions (235 ± 20 ms) for both men and women. During recovery, the silent period remained elevated for the stressor session relative to control and mental-attentiveness sessions (quadratic interaction of time × session, $P < 0.05$).

Silent period during 20% fatiguing contraction. The silent period at the end of the 20% fatiguing contraction was similar for men and women and had similar increases from baseline to task failure during the 20% contraction for the control and stressor sessions and control and mental-attentiveness sessions ($P > 0.05$).

Contractile Properties

Estimated resting twitch. Estimated resting twitch was similar at baseline for the control, stressor and the mental-attentiveness session (session effects for each analyzed separately, $P > 0.05$). Men had greater resting twitch amplitudes than women at baseline and after the fatiguing contraction (sex effect, $P < 0.001$) for all three sessions. The amplitude of the twitch decreased after the fatiguing contraction similarly for the control and stressor session (session × fatigue, $P = 0.12$) and for men and women (fatigue × sex, $P = 0.18$, Figure 3.4C)**.** There were no interactions ($P > 0.05$). The mental-attentiveness task did not have an effect on the estimated resting twitch ($P > 0.05$).

Peak relaxation rates. The peak rates of relaxation were similar at baseline between the control and stressor sessions (session effect at baseline, $P = 0.92$). Men had faster peak relaxation rates than women before and after the fatiguing contraction (sex effect, $P = 0.004$). Peak rates of relaxation were not different after the fatiguing contraction (fatigue effect, $P = 0.33$) for either session (session × fatigue, $P = 0.13$) for men and women (session × fatigue × sex, $P = 0.88$). Peak rates of relaxation increased after the fatiguing contraction throughout recovery up to 20 min (fatigue effect during recovery, $P = 0.005$, Figure 3.4D) for both men and women (fatigue × sex, $P = 0.09$). The peak relaxation rates (average of both sessions) were correlated with the maximal strength (average of both sessions, $r = -0.48$, $r^2 = 0.23$, $P = 0.01$). Those who were stronger had faster rates of relaxation. The baseline peak relaxation rates were also correlated with the time to failure for the control session ($r = 0.46$, $r^2 = 0.21$, $P = 0.02$) so that individuals with a faster peak rate of relaxation had a briefer time to task failure. The association was not significant for the stressor session ($r = 0.27$, $r^2 = 0.07$, $P = 0.16$). The

change in peak relaxation rate from the control to the stressor session was also not correlated with the change in time to failure when exposed to the cognitive stressor (P = 0.72). Mental-attentiveness task did not have an effect on the peak relaxation rates.

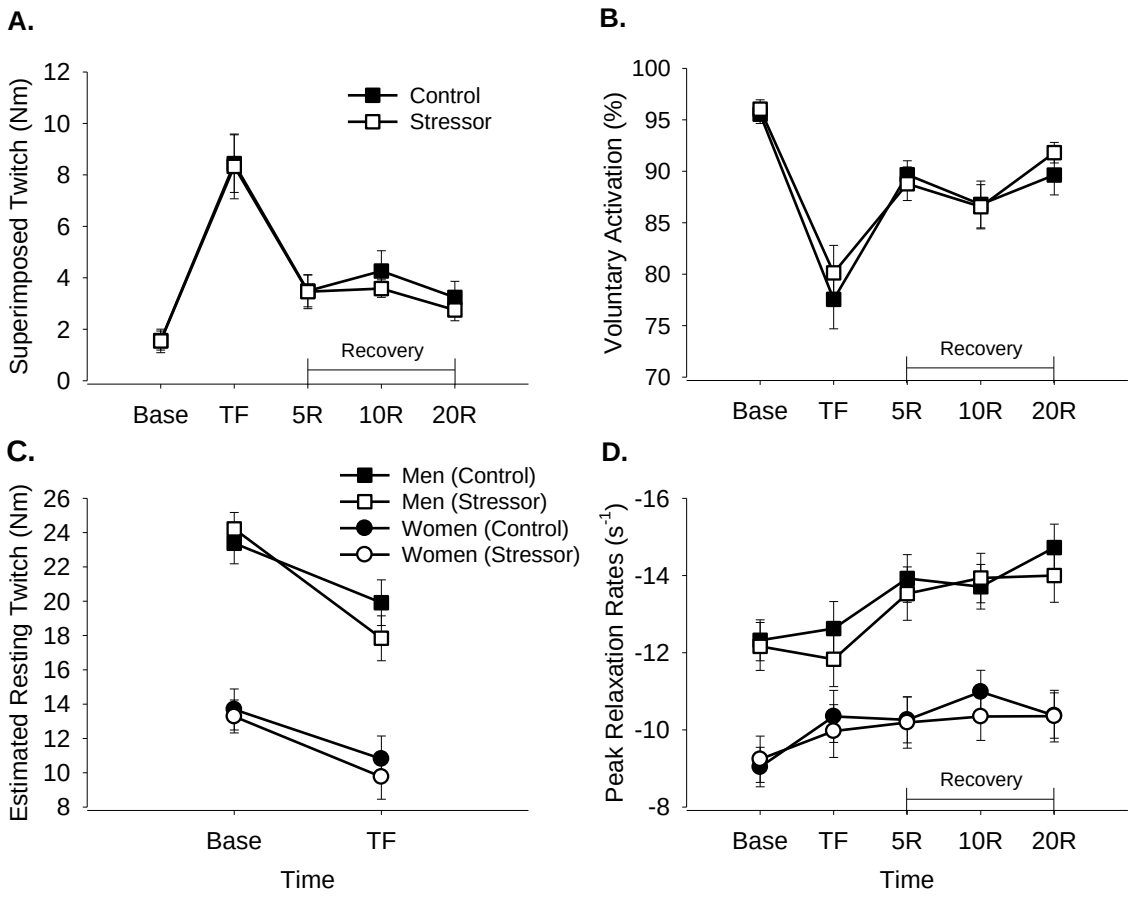

Figure 3.4. Superimposed Twitch (SIT) (*A*), Voluntary Activation (*B*), Estimated Resting Twitch (*C*) and Peak Relaxation Rates (*D*) for the Biceps Brachii for the Control and Stressor Session. Superimposed twitch and voluntary activation was similar for the control and stressor sessions before, immediately after the fatiguing contraction and throughout recovery ($P > 0.05$). Estimated resting twitch and the peak relaxation rates were greater and faster (respectively) for men than women ($P < 0.05$) but were not affected by the cognitive stressor ($P > 0.05$). Values are represented as the mean ± SE. Base indicates baseline measures before the fatiguing contraction, TF indicates measures recorded immediately upon task failure and 5R, 10R and 20R indicates 5, 10 and 20 minutes of recovery respectively.

Cardiovascular Responses to Stressor and Fatigue

Cardiovascular responses to stressor prior to the fatiguing contraction. Heart rates were similar at baseline for both the control and stressor session (session effect, P =

0.17) with no effect of sex ($P = 0.99$). Heart rate was greater during the 4 minutes of mental math (stressor session) compared with the quiet sitting (control session) (81.9 ± 2.7 vs. 76.7 ± 2.5 $b \cdot min^{-1}$, respectively, session effect, $P = 0.04$). There was no effect of sex ($P = 0.59$) and no interactions ($P > 0.05$). MAP was similar at baseline across sessions ($P = 0.17$) for both men and women (session × sex, $P = 0.79$) and increased by 30 s into the mental-math task (stressor session) compared with quiet sitting (control session) (session × time, $P = 0.002$). MAP remained elevated during the 4 minutes of mental math compared with the quiet sitting (101.3 ± 4.8 vs. 83.9 ± 0.8 mmHg, respectively, session effect, $P < 0.001$). MAP response was greater for the men compared with the women during the stressor session (session × sex, $P = 0.02$).

Cardiovascular response during the fatiguing contraction. Heart rate increased throughout the fatiguing contraction from baseline to task failure (time effect, $P < 0.001$) differently for the control and stressor sessions (session × time, $P = 0.03$, Figure 3.5A). Heart rates increased in the beginning of the task during the stressor session compared with the control session, but were similar with the control session at the end of the task. There was no effect of the mental-attentiveness task on heart rate during the fatiguing contraction (session effect, $P = 0.53$). Men had greater increases in heart rate during the fatiguing contraction (time × sex, $P = 0.007$) and overall greater heart rates for both control and mental-attentiveness sessions (sex effect, $P = 0.01$, Figure 3.6A).

MAP increased throughout the fatiguing contraction from baseline to task failure (time effect, $P < 0.001$) at a greater rate during the stressor session compared with the control session for both men and women (session × time, $P = 0.02$). MAP was greater for men compared with women (sex effect, $P < 0.001$) and men had greater rates of increase

throughout the fatiguing contraction for both the control and stressor sessions (time × sex, $P = 0.008$, Figure 3.5B). MAP was not affected by the mental-attentiveness task (session effect, $P = 0.32$) but MAP was greater for men than women for both the control and mental-attentiveness sessions (sex effect, $P = 0.004$, Figure 3.6B).

To understand the cardiac workload during the fatiguing contraction, rate pressure product was quantified (heart rate x MAP) (Wasmund et al., 2002). The rate pressure product increased throughout the fatiguing contraction (time effect, $P < 0.001$) at a greater rate for the stressor session compared with the control (session × time, $P = 0.006$) and more so for the men than the women for both sessions (time × sex, $P = 0.02$, Figure 3.5C).

Rating of Perceived Exertion

Rating of perceived exertion (RPE) increased at a greater rate during the fatiguing contraction in the control session compared with the stressor session (session × time, $P = 0.004$) with an overall effect of session ($P < 0.001$). Increases were similar for men and women for both sessions (time × sex, $P = 0.08$). Similar to the stressor session, the RPE during the mental-attentiveness task was overall less than the control session (session effect, $P = 0.02$). There were no significant interactions ($P > 0.05$) and no effect of sex ($P = 0.81$) between the control and mental-attentiveness sessions.

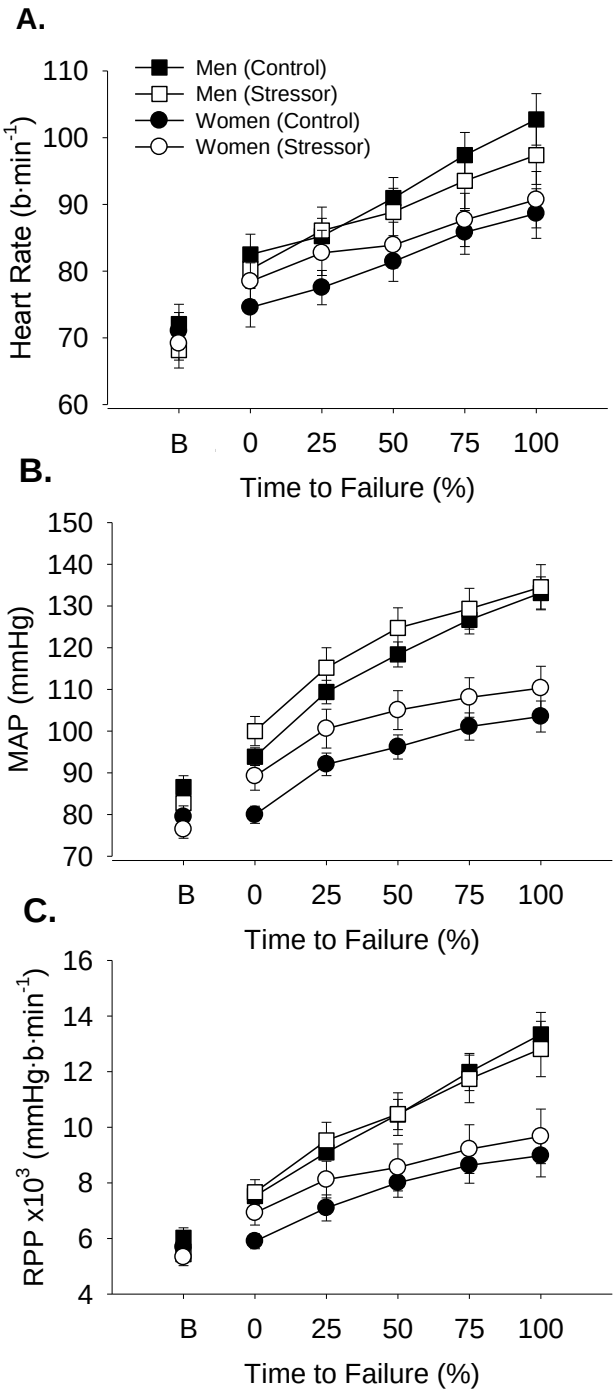

Figure 3.5. The Heart Rate *(A)*, Mean Arterial Pressure (MAP;(*B*)) and Rate Pressure Product (RPP;(*C)*) for Control and Stressor Sessions. Heart rate, MAP and the RPP were all greater throughout the stressor session compared with the control session ($P < 0.05$).The values are presented as mean ± SE at 25% increments of the time to task failure for men (squares) and women (circles). B indicates baseline measures for each variable. Averages of 15-s intervals were used for the MAP and heart rate.

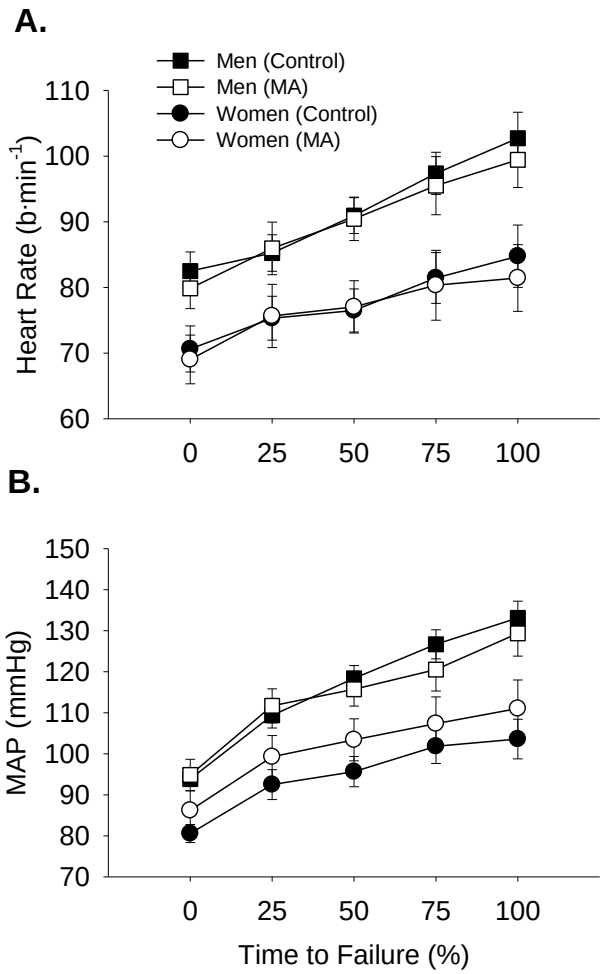

Figure 3.6. The Heart Rate *(A)* and Mean Arterial Pressure (MAP;(*B*)) for Control and Mental-attentiveness (MA) Session. Heart rate and MAP were similar for control and mental-attentiveness sessions ($P < 0.05$).The values are presented as mean ± SE at 25% increments of the time to task failure for men (squares) and women (circles). Averages of 15-s intervals were used for the MAP and heart rate.

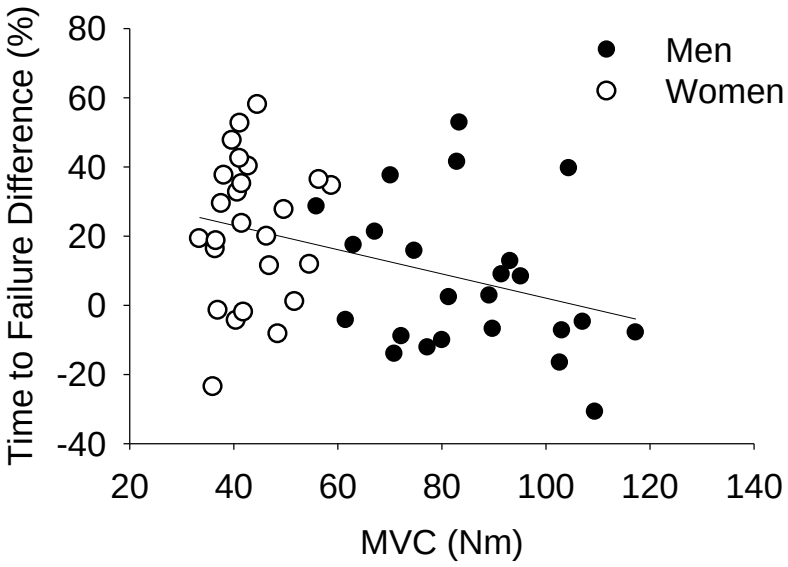

Figure 3.7. Association between MVC Torque and Time to Failure Difference (%). This figure demonstrates combined results from the previous study (Yoon et al. 2009) with the present study (n = 48) demonstrating an association between the relative difference in time to failure between the control and stressor session and the initial strength of the individual. MVC torque was the mean of the control and stressor session. Initial MVC torque was negatively correlated with the relative and absolute difference in time to task failure between the control and stressor session (relative difference: $P = 0.005$, $r = -0.39$, $r^2 = 0.15$ and absolute difference: $P = 0.004$, $r = -0.40$, $r^2 = 0.16$, statistics for graph are for combined studies). Correlations were not significant when performed separately for men and women (men: $r = -0.31$, $P = 0.15$ and women: $r = 0.08$, $P = 0.70$).

DISCUSSION

This study investigated sex differences in neural and muscular mechanisms that may contribute to decrements in muscle fatigability when exposed to a cognitive stressor (difficult mental-math task) during a low-intensity contraction with the elbow flexor muscles. Time to task failure was reduced for both men and women when exposed to a cognitive stressor and this decrement tended to be larger for the women. The novel findings were that the greater fatigability with the cognitive stressor was not due to greater decrements in neural drive from supraspinal sources, nor due to differences in corticomotor excitability or muscle properties reflected by peak relaxation rates. The difference in time to task failure correlated with the strength of the individual, such that

weaker subjects had greater reductions in time to failure during the stressor session compared with control. The reduction in strength was similar across sessions in both men and women despite the decrease in time to failure during the stressor session, demonstrating that this decrement was not due to premature termination of the sustained contraction. Furthermore, the reduction in time to failure with the difficult mental-math task was not due to distraction because a mental-attentiveness task did not influence time to failure, supraspinal fatigue or the peak relaxation rates of the muscle.

Psychosocial and Physiological Responses induced by the Stressor

An increase in psychosocial stress (arousal) can result in activation of the sympathetic nervous system with subsequent release of neuromodulators and hormones throughout the central and peripheral nervous system (McEwen, 2000), causing elevations of heart rate, blood pressure and cortisol levels (Yoon et al., 2009). In our study, both men and women had elevated measures of arousal when assessed with the mental math (stressor task) at rest. Perceived anxiety (VAS and STAI-state), heart rate and blood pressure were all elevated during the stressor task at rest relative to quiet sitting (control session) indicating that sympathetic activation was elevated. There was however, a sex difference for some of these responses. Women for example, reported higher levels of anxiety (VAS) than men at baseline and after the stressor, however heart rate did not differ between the sexes even though women reported feeling more anxious. In contrast, men had greater increases in MAP than women during the 4 minutes of mental math. The reason is not apparent but could have been due to men inadvertently activating mechanoreceptors that contribute to the increase in blood pressure (Rowell & O'Leary, 1990) during the task while at rest. Also, the greater increase in anxiety (VAS)

during the stressor session relative to control also was apparent after the fatiguing contraction for both men and women. These results indicate that the mental-math task used to induce stress was strong enough to generate differences in arousal between sessions before and during the fatiguing contraction.

Supraspinal Fatigue and Corticomotor Excitability during Contraction was not Influenced by the Cognitive Stressor

Voluntary activation measured with superimposition of a stimulus at the cortex during a maximal effort reflects the ability of the motor cortex to maximally drive the distal nervous system and muscle. Any deficit in voluntary activation indicates that the recruitment of motor units and/or discharge frequencies were not optimal during the maximal effort (Gandevia, 2001). Prior to the fatiguing contraction, voluntary activation for men and women was near maximal (96% for both sessions) and similar for the sexes. Voluntary activation elicited with TMS was reduced to ~79 % for men and women after the low-intensity fatiguing contraction as seen before (Smith et al., 2007; Yoon et al., 2007) and remained depressed even 20 minutes after termination of the sustained contraction. Thus, supraspinal fatigue contributed to the loss of MVC force after the low-force fatiguing contractions similarly for both men and women as seen before (Chapter II). Supraspinal fatigue however, was similar at task failure and during 20 minutes recovery for both the control and stressor sessions. Furthermore, the difference in time to failure between the stress and control session was not associated with voluntary activation or any difference in voluntary activation between sessions. Thus, although the increase in physiological stress response can be centrally mediated and different for men

and women (Valentino & Van Bockstaele, 2008), increased arousal did not appear to influence neural drive immediately after a low-force fatiguing contraction.

Corticomotor excitability was quantified with the MEP during voluntary contractions. The MEP is the EMG response elicited from the TMS and represents the net excitation in the corticomotor tract (Taylor & Gandevia, 2001). Biceps brachii and brachioradialis MEP area increased during and immediately after the fatiguing contraction, indicating an increase in the excitability within the central nervous system (Taylor & Gandevia, 2001). This increase was similar for both men and women and across sessions. The compound muscle action potential (M wave) which can contribute to the MEP (Taylor et al., 1999), was similar throughout the fatiguing contraction and across sessions. Consequently, propagation of the action potential at the neuromuscular junction (Taylor et al., 1999) did not change with muscle fatigue or arousal in men and women. The increase in MEP therefore, may have been due to an increase in cortical excitability or motoneuron excitability because both MEP and responses to stimulation of descending tracts at the cervicomedullary (CMEP) level increase with fatigue during submaximal contractions of the elbow flexor muscles (Levenez et al., 2008). Nevertheless, the increase in corticomotor excitability measured during voluntary contractions was similar across sessions and could not explain the increase in fatigability when exposed to the stressor.

The low-intensity fatiguing contraction resulted in a longer silent period duration of the EMG and the increase was similar across sessions. The silent period with fatigue has traditionally thought to reflect inhibition in the spinal cord (early phase) (Inghilleri et al., 1993) and also cortical inhibition from interneurons (mainly $GABA_B$-mediated) in the

latter phase (Inghilleri et al., 1996). More recent studies demonstrate that the increase in inhibition may be mediated primarily by spinal mechanisms during maximal (McNeil et al., 2009) and low-intensity submaximal contractions (McNeil et al., 2011). The silent period duration elicited during the MVC however, was greater at baseline for the cognitive sessions compared with control indicating greater inhibition at the start of the stressor session. Anticipation may have played some role in the longer silent period although baseline measurements of the silent period occurred prior to the participants having knowledge of which session they were attending (at least for their first session). Thus by deduction, some subjects may have been aware that a cognitive session was to occur prior to the measurements. Despite reaching similar silent period durations at task failure, the silent period remained elevated in early and mid-recovery for the stressor session relative to the control session. Difficult mental activity or anticipation of the task may have resulted in some corticomotor inhibition. The greater silent period duration at baseline and during recovery for the cognitive sessions is not understood and deserves exploration in future studies. Taken together, changes within the motor cortex as measured by TMS during voluntary contractions did not reveal any neural mechanisms that contribute to greater fatigability when young adults were exposed to a cognitive stressor.

Muscular mechanisms of neuromuscular fatigue and stress

Consistent with our previous study (Yoon et al., 2009), we demonstrated that the greater fatigability during the stressor session was associated with the strength of the individual. In the current study, the time to failure was reduced with the stressor by 21.6 % for women and 10.6% for men. This combined with our previous data demonstrates an

association between initial strength and the difference between sessions in time to failure (r^2 = 0.16, see Figure 3.7). The associations with strength could be due to one of two mechanisms that are mediated by an increase in sympathetic activation: (1) fiber types and (2) stress-induced changes in blood perfusion that have greater effects in weaker subjects (Yoon et al., 2009). In this study we investigated whether the peak relaxation rates, which reflect the greater proportion of Type II fibers in men and are associated with strength, mediate the changes in time to failure with stress. Contractile force can be potentiated in Type II fast-twitch fibers but reduced in Type I slow-twitch fibers in animal and human muscle when sympathetic activation is high (Bowman, 1980; Roatta et al., 2008) predisposing muscle with more Type I fibers to increased fatigability with sympathetic activation. We found that men exhibited greater estimated resting twitch amplitudes and faster peak relaxation rates than women before and after the fatiguing contraction. Faster relaxation of evoked contractions is consistently associated with greater proportions of Type II fibers and faster calcium uptake into the sarcoplasmic reticulum in human muscle (Hunter et al., 1999). Any stress-induced sympathetic activation however, did not alter the peak relaxation rates or estimated resting twitch for men or women (see Figure 3.4C and 3.4D). Accordingly, the peak relaxation rates were associated with time to failure for the control session but there was no association between these two variables for the stressor session. Furthermore, there was no association between the change in peak rates of relaxation and the difference in time to task failure between sessions. Thus, the change in time to failure with stress was likely not due to a differential activation of Type I and Type II fibers in subjects of different strength. Although peak relaxation rates increased for both men and women throughout

recovery for both sessions as seen before (Chapter II and Kuchinad et al., 2004), this was likely due to increased muscle temperature (Todd et al., 2005) during the fatiguing contraction.

Alternatively, the strength-related reduction in time to failure with the stressor may have been mediated by perfusion differences between strong and weaker subjects. Women, who were weaker in this study, typically have lower blood pressure (Wiinberg et al., 1995), a blunted vasoconstrictor response to α-adrenergic stimulation in the brachial artery (Kneale et al., 2000) and lower muscle sympathetic nerve activity (MSNA) (Ettinger et al., 1996). During a submaximal contraction in the absence of psychosocial stress (control), perfusion of the muscle and the time to task failure can depend on muscle size and absolute strength. Weaker individuals (both men and women) for example can have greater perfusion during a submaximal contraction compared with stronger men and women (Barnes, 1980; Hunter et al., 2006b) and therefore a longer time to task failure under control conditions (Hunter, 2009). Accordingly, women who are usually weaker than men, have a slower rate of rise in MAP during a submaximal contraction which likely reflects less of a build-up of metabolites (less metaboreflex) in women than men (Hunter & Enoka, 2001). Increased arousal however, can increase sympathetic activation and alter the balance of vasoconstriction and vasodilation (Halliwill et al., 1997). This balance in perfusion potentially differs in response to arousal between strong and weak subjects because weaker subjects have greater perfusion at baseline. Indirect indicators of sympathetic activation (MAP and heart rate) were elevated during the stressor session compared with the control session. The mechanism for the greater fatigability therefore

may be due to the stress-induced sympathetic activation and increased vasoconstriction to the weaker muscles when exposed to the stressor.

The increase in blood pressure and heart rate with exposure to the stressor during the fatiguing contraction also resulted in increased cardiac load which was indicated by the rate pressure product. The rate pressure product was greater for the men than the women for both the control and stressor session but both sexes had increased cardiac load with the stressor. These findings can have significant clinical implications for both men and women who perform low-force sustained contractions for prolonged periods under stressful work conditions.

Conclusion

Our study indicates that in young adults, the increased fatigability that occurs with increased stress is associated with initial strength and is not due to muscular mechanisms associated with peak relaxation rates, or neural mechanisms associated with neural drive or corticomotor excitability. Mental distraction also did not contribute to the increased fatigue because time to failure was similar for the control and mental-attentiveness sessions. Because the increased fatigability was associated with initial strength, the mechanism may involve stress-related changes in muscle perfusion and blood flow regulation in weaker young adults during low-intensity sustained contractions.

Chapter IV

Sex Differences in Motor Output Variability with Fatigue and Stress for Low-Intensity Contractions

SUMMARY

The purpose of this study was to compare the effect of fatigue on force fluctuations for a very low-intensity contraction (5% of maximal voluntary contraction, MVC) with the elbow flexor muscles in the presence and absence of a cognitive stressor. Twenty-eight young adults (14 women and 14 men, 20 ± 3 years) participated in experimental sessions (control and stressor sessions). A subset of the subjects (14 men, 9 women) participated in a third experimental session (mental-attentiveness). Subjects performed brief contractions at 5% MVC before and after a difficult mental-math task (stressor), a simple mental-math task (mental-attentiveness) or quiet sitting (control) and during recovery from a fatiguing contraction, when these tasks were performed simultaneously during the contraction. Anxiety (State Trait Anxiety Inventory), heart rate, mean arterial pressure and EMG was assessed to throughout the very low-intensity contractions and fatiguing contraction. Measurements of anxiety (State Trait Anxiety Inventory) increased after exposure to the stressor ($P < 0.05$). The amplitude of force fluctuations increased from baseline to after the difficult mental-math task (stressor) ($P < 0.05$) and after the mental-attentiveness task ($P < 0.05$) for the women but not for the men. Force fluctuations (% CV) increased immediately after the fatiguing contraction ($P < 0.001$) similarly for the control and stressor session ($P > 0.05$) and men and women (P

> 0.05). Women however, had greater force fluctuations compared with men throughout the 20 min of recovery after the fatiguing contraction ($P < 0.001$). The greater force fluctuations in women were not due to greater indices of neural or sympathetic activation, quantified by heart rate, mean arterial pressure or EMG activation patterns.

INTRODUCTION

Motor output variability (steadiness) of a low-intensity contraction is an important determinant of motor function and is associated with performance of functional tasks in men and women (Marmon et al., 2011). While maintaining a steady contraction for example, the force exhibited by the muscle fluctuates about a target force and this represents the variability in motor output. The magnitude of fluctuations can be quantified as the standard deviation of the force signal normalized to its mean (coefficient of variation, CV) (Galganski et al., 1993) Several factors that can influence the variability in motor output include the age and sex of the individual (Noteboom et al., 2001b; Christou et al., 2004; Brown et al., 2010), the muscle group (Galganski et al., 1993; Graves et al., 2000; Tracy & Enoka, 2002), the contraction type and intensity (Galganski et al., 1993; Keen et al., 1994; Laidlaw et al., 1999; Laidlaw et al., 2000), the state of arousal (stress) (Noteboom et al., 2001a; Christou et al., 2004) and neuromuscular fatigue (Cresswell & Loscher, 2000; Hunter & Enoka, 2001; Hunter et al., 2004c).

Differences in motor output variability between populations, muscle group and state of arousal in general are greater during low-intensity contractions when Type I (slow) motor units are predominantly recruited (Enoka et al., 2003; Christou et al., 2004; Brown et al., 2010). Women for example, who are usually weaker and possess a greater

proportion of Type I fibers (Simoneau & Bouchard, 1989; Roepstorff et al., 2006) were less steady (greater force fluctuations) than men for low and high-intensity contractions, but the greatest sex difference appears to be at the very low-intensity contractions (≤ 10% of MVC) (Brown et al., 2010).

In the absence of fatigue, sex differences in force fluctuations for very low-intensity contractions with hand muscles can be exacerbated when arousal (stress) is increased (Noteboom et al., 2001b; Christou et al., 2004). The mechanism for increased force fluctuations with arousal is not understood but may involve differences in the activation of the sympathetic nervous system that can alter the input-output gain of the motoneuron pool (Heckmann et al., 2005) or reduce muscle spindle sensitivity which can alter motoneuron characteristics (Roatta et al., 2002). Furthermore, the mechanisms for the sex differences in force fluctuations when exposed to a stressor are not understood.

Neuromuscular fatigue will also increase motor output variability (Cresswell & Loscher, 2000). As fatigue develops, the motoneuron pool receives less excitatory (or more inhibitory) afferent input due to an increase in feedback transmitted by chemically sensitive Type III and IV afferents (Bigland-Ritchie et al., 1986; Garland et al., 1994) and a reduction in feedback from stretch-sensitive afferents (Duchateau et al., 2002). Potential contributions to increased force fluctuations with fatigue therefore, are an increased Ia afferent input to the alpha motoneuron pool (Cresswell & Loscher, 2000), increased synchronization of motor units (Holtermann et al., 2009) (which is likely to be greater among motor units with smaller and slower twitches) (Schmied et al., 1993; Schmied et al., 1994) and coactivation of the antagonist (Vallbo & Wessberg, 1993; Spiegel et al., 1996).

Arousal potentially augments the increase in motor output variability with fatigue. We recently demonstrated similar increases in force fluctuations during a fatiguing contraction at a low-to-moderate intensity (20% of MVC) for both young men and women when performing a difficult mental-math task (which increased arousal) (Yoon et al., 2009). Whether cognitive stress and fatigue influence the sex difference in force fluctuations for very low-intensity (5% of MVC) contractions, where sex differences are the greatest and potentially most important for functional performance (Marmon et al., 2011), is unknown.

The aim of this study therefore, was to determine the sex difference in force fluctuations for a very low-intensity contraction (5% of MVC) with the elbow flexor muscles before and after a fatiguing contraction that was performed in the presence and absence of a cognitive stressor. We hypothesized that women would have greater increases in force fluctuations compared with men for a 5% MVC task after exposure to the stressor and fatigue combined. To fatigue the muscles, subjects performed a sustained contraction at 20% of MVC until task failure in the presence and absence of a cognitive stressor. To provide insight to possible mechanism, physiological variables including heart rate, mean arterial pressure and electromyography (EMG) were measured.

METHODS

Twenty-eight young adults (14 women, 14 men, 20 ± 3 years) participated in a familiarization session and two experimental sessions (control and stressor sessions) to perform low-intensity contractions with the elbow flexor muscles of the left arm. Contractions were performed prior to and after a cognitive stressor (mental-math task) and after a fatiguing contraction. A subset of the subjects participated in a third

experimental session (mental-attentiveness task) (14 men, 20 ± 2 years and 9 women, 20 ± 4 years) to determine whether performance of a non-stressful cognitive task (mental attentiveness) would influence force fluctuations for a very low-intensity contraction. The order of the experimental sessions was counterbalanced among the subjects and the experimental sessions were ≥7 days apart. Prior to participation in the study, each subject provided informed consent. The protocol was approved by the institutional review board at Marquette University.

All subjects were healthy with no known neurological or cardiovascular diseases and were naive to the protocol. Subjects reported no history or current mental pathology, including anxiety and/or depressive disorder. Levels of anxiety were quantified by the Trait STAI during the familiarization session and the State STAI before and after exposure to the cognitive stressor (Spielberger, 1970). For each female participant, the day of their menstrual cycle was reported for that experimental day. The first day of menstruation was considered as day one of the cycle.

Mechanical Recordings

Each subject was seated upright in an adjustable chair with the left arm slightly abducted and the elbow joint resting on a padded support. The elbow joint was flexed to 90 degrees in order for the forearm to be horizontal to the ground and the force at the wrist was directed upward with activation of the elbow flexor muscles. The hand and forearm were placed in a modified wrist-hand-thumb orthosis (Orthomerica, Newport Beach, CA) and the forearm was placed in the neutral position. Two nylon straps were placed over each shoulder to minimize shoulder movement. The force exerted by the wrist in the vertical direction was measured with a transducer (JR-3 Force-Moment

Sensor; JR-3 Inc., Woodland, CA) that was mounted on a custom-designed, adjustable support. The orthosis was attached to the force transducer. The force was recorded on-line at 500 samples·s^{-1} using a Power 1401 A-D converter and Spike 2 software (Cambridge Electronic Design, Cambridge, UK) and displayed on a 19-inch monitor 1.5 m in front of the subject. Each subject was asked to trace the horizontal cursor with the force signal for as long as possible during the fatiguing contraction. The force signal appeared on the screen from the right side of the monitor at 2.5 cm·s^{-1}. Force fluctuations were quantified as a measure of motor output variability. The amplitude of the force fluctuations was quantified as the coefficient of variation of the force (CV = SD/mean × 100) (Galganski et al., 1993)

Electrical Recordings

EMG signals were recorded with bipolar surface electrodes (Ag-AgCl, 8-mm diameter; 16 mm between electrodes) that were placed over the long head of the biceps brachii, brachioradialis, and long head of the triceps brachii muscles. The bipolar electrode configuration was placed longitudinally over the muscle belly midway between the origin and insertion for each muscle, according to the European recommendations for surface EMG (Hermens et al., 2000). Reference electrodes were placed on a bony prominence at the elbow. The EMG signal was amplified (100×) and band-pass filtered (13-1000 Hz) with Coulbourn modules (Coulbourn Instruments, Allentown, PA) prior to being recorded directly to a computer with the Power 1401 A-D converter and Spike 2 (CED). The EMG signals were digitized at 2000 samples·s^{-1}.

Cardiovascular Measurements

Heart rate and blood pressure were monitored at rest (baseline), during the cognitive tasks or quiet sitting (prior to the fatiguing contraction) and also during each fatiguing contraction. Both heart rate and blood pressure were monitored with an automated beat-by-beat, blood pressure monitor (Finapres 2300; Ohmeda, Louisville, CO). The blood pressure cuff was placed around the middle finger of the relaxed, right hand with the arm placed on a table adjacent to the subject at heart level. The automated blood pressure signal was calibrated to a manual blood pressure reading for each participant. The blood pressure signal was recorded on-line to a computer at 500 samples$\cdot s^{-1}$.

Cognitive Assessment of Arousal

The STAI-state questionnaire involved 20 statements that required a response on a four-point, Likert-type scale. Assessment of STAI was performed at baseline and after quiet sitting (control session) and after 4 minutes (2 x 2-min bouts) of simple and difficult mental-math tasks (cognitive sessions) (Figure 4.1).

Cognitive Tasks

Mental math is an established psychosocial technique to induce stress (Kajantie & Phillips, 2006) that we have used to increase levels of anxiety (Yoon et al., 2009). Each subject was asked to perform serial subtraction from a 4-digit number by 13 with a response required every 3 s (Noteboom et al., 2001b). Once the subject made an error in the math or was not able to provide the correct answer within 3 s, they were asked to start the mental math again from a new number in the series. Each subject performed the mental math during the mental-math experimental session only. They performed the

mental math before the fatiguing contraction (2 x 2 min bouts) and then continuously during the fatiguing contraction. This particular mental-math task was chosen because it has been well established to increase levels of arousal (Noteboom et al., 2001b).

The mental-attentiveness task required subjects to perform a simple mental-math task that was not designed to induce stress. Participants subtracted by one from 50 continuously during the 4-minutes (2 x 2 min bouts) prior to the fatiguing contraction and during the fatiguing contraction in the mental-attentiveness session. During the control session, no cognitive tasks were performed: each subject sat quietly for the 2 x 2-min bouts and performed the fatiguing contraction with no cognitive tasks.

Experimental Protocol at Experimental Sessions

All procedures were performed in the following order for each experimental session (Figure 4.1): (1) baseline assessments of cognitive and physiological arousal, (2) MVC of the elbow flexor and extensor muscles, performance of brief 5% of MVC contractions before and after either quiet sitting (control session) or 4 minutes (2 x 2-min bouts) of cognitive tasks (mental-math and mental-attentiveness sessions only), (3) cognitive arousal response (STAI) to cognitive tasks or quite sitting, (4) performance of a fatiguing contraction at 20% MVC force, (5) MVCs and 5% contractions immediately following task failure and at 5, 10 and 20 minutes recovery.

Pre-fatigue measures. Three to four elbow flexor MVCs were performed to determine if the subject was within 5% of previous sessions and to calculate force for the steadiness contractions at 5% MVC and submaximal fatiguing contraction at 20% MVC. Two MVCs of the elbow extensors were performed so that peak EMG values could be obtained to normalize the triceps EMG activity during the fatiguing contractions.

Fatiguing contraction. A fatiguing contraction was performed with the elbow flexor muscles at 20% MVC force during each experimental session. The subject was required to match the vertical target force as displayed on the monitor and was verbally encouraged to sustain the force for as long as possible. The fatiguing contraction was terminated when the target force declined by 10%. To minimize the influence of transient fluctuations in motor output on the criteria for task failure, the task was terminated only after force fell below the predetermined threshold for 2 out of a 4-s interval. Task failure was detected automatically using a custom-designed program (Spike 2, CED) that monitored the force signal and this time was recorded as the time to task failure.

Recovery measures. Recovery measure of 5% MVC force contractions were assessed at the following times: immediately upon task failure, 5, 10 and 20 minutes after termination of the fatiguing contraction.

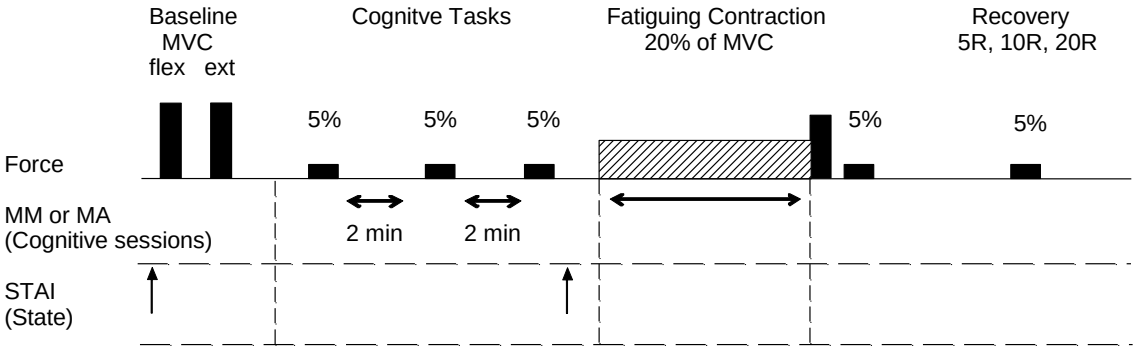

Figure 4.1. Experimental Protocol. The top panel shows the order of force tasks performed by each subject. Maximal voluntary contraction (MVC) with the elbow flexors were performed followed by MVCs of the elbow extensors. Brief contractions at 5% of MVC, a fatiguing contraction at 20% MVC and then recovery MVCs and 5% brief contractions with the elbow flexor muscles were performed. Mental-attentiveness (MA) and mental-math (MM) were performed 2 x 2 min (total of 4 min) before the fatiguing contraction and then during the fatiguing contraction for each respective session. State-Trait Anxiety Inventory (STAI) questionnaire was assessed twice throughout the protocol. The bottom panel shows the order in which the events took place (5R, 10R and 20R denotes recovery at 5, 10 and 20 minutes respectively). Note that the schematic is not to scale for time or force.

Data Analysis

The MVC force was quantified as the average value over a 0.5-s interval that was centered about the peak of the MVC. The torque for the MVC and submaximal contractions was calculated as the product of force and the distance between the elbow joint and the point at which the wrist was attached to the force transducer. The maximal EMG for each muscle was determined as the root mean squared (RMS) value over a 0.5 s interval about the same interval of the MVC torque measurement. The maximal EMG value for the biceps brachii, brachioradialis and triceps brachii was then used to normalize the RMS EMG values recorded during the fatiguing contraction for each respective muscle. Coactivation of the agonist and antagonist muscles (biceps and triceps) was calculated by normalizing the agonist EMG muscle activity to the antagonist EMG muscle. The RMS EMG of the elbow flexor muscles, triceps brachii muscles, coactivation of EMG and the fluctuations in force (CV of force) were quantified during the fatiguing contraction at the following time intervals: the first and last 30 s of task duration and 15 s either side of 25%, 50%, and 75% of time to failure. Force fluctuations, RMS EMG activity (biceps brachii and brachioradialis), heart rate and mean arterial pressure were also quantified during the 5% contractions several times throughout the sessions. Contractions were held for approximately 10 s and these variables were measured and quantified for a duration of 4 s during each 10 s contraction. Measurement began 4 s after the start of the contraction. Triceps brachii EMG activity and therefore coactivation was not able to be quantified in the 5% contraction force due to the artifact in the triceps EMG signal.

To quantify the bursts of EMG activity, the rectified EMG signal was smoothed with a low-pass filter at 2 Hz over 5-point averages and divided by the average of the rectified EMG so that muscles with different EMG amplitudes could be compared. The differentiated signal represents the rate of change for the low-pass–filtered EMG signal and was used to identify rapid changes in the EMG signal. A burst was identified when the smoothed, differentiated EMG signal increased by > 0.20 s^{-1}. These values represented 3 SD above the mean of the smoothed, differentiated EMG signal. The 3-SD criterion was based on EMG records from fatiguing contractions of the present data set when the EMG signal displayed minimal bursting during the contraction. The end of a burst was identified as the time when the smoothed EMG signal decreased to the same amplitude as at the start of the burst. When this failed to occur, however, the end of the burst was identified as the time when the differentiated EMG signal became most negative prior to the start of the next burst. This criterion represented the time at which the signal decreased most rapidly before the beginning of the next burst.

During the mental-math task (stressor) or quiet sitting, heart rate and MAP were analyzed at the start of the first 2 min bout, and then at 30 s and 90 s after the start of each 2 min bout of the mental task. During the mental-attentiveness task, heart rate and MAP (prior to the fatiguing contraction) were unable to be analyzed for seven (2 men and 5 women) individuals due to artifact in the signal. This data is therefore not reported. Heart rate and MAP were also recorded during the fatiguing contraction and analyzed by comparing ~15s averages at 25% intervals. For each interval, the blood pressure signal was analyzed for the mean peaks [systolic blood pressure (SBP)], mean troughs [diastolic blood pressure (DBP)], and number of pulses per second (multiplied by 60 to determine

heart rate). MAP was calculated for each epoch with the following equation: MAP = DBP +1/3 (SBP – DBP).

During the stressor session the errors were calculated for the mental math prior to the fatiguing contraction separately from the errors from those that were made during the fatiguing contraction. Error rate was calculated from the errors that were made divided by the duration of time of the mental math, i.e. # of errors/4-minutes (stressor task only) and # of errors/time to failure (minutes) for the fatiguing contraction.

Statistical Analysis

Data are reported as means ± SD within the text and displayed as means ± SEM in the figures. Two separate analyses were completed for this study because not all subjects returned to complete the mental-attentiveness session. Therefore, two-way ANOVAs with repeated measures over time and sex as a between-subject factor (men vs. women) were used to compare the various dependent variables for the control and stressor session and then for the control and mental-attentiveness session. P values were exact if results were reported for one analysis. If results were similar for both analyses, then results were reported together and *P* values were reported as greater or less than 0.05. Repeated measures factors included session (control and stressor session and control and mental-attentiveness session), fatigue (baseline, after fatiguing task and recovery) and time (0, 25, 50, 75, and 100% of time to failure). Specifically, the statistical designs were as follows for the dependent variables: (1) session × fatigue × sex for comparison of force fluctuations (CV), biceps and brachioradialis RMS EMG, heart rate and MAP during the 5% contraction from baseline to after the fatiguing contraction; (2) session × time × sex for levels of anxiety (STAI) throughout the session, RMS EMG,

force fluctuations, heart rate and mean arterial pleasure after the initial cognitive task (or quiet sitting) and time throughout recovery (3) session × time × sex for force fluctuations, RMS EMG, bursting EMG, coactivation, MAP and heart rate during the fatiguing contraction and heart rate and mean arterial pressure during the cognitive tasks (prior to the fatiguing contraction).

An independent t-tests was used to compare men and women for various physical characteristics such as, age, weight, height, handedness (refer to Table 3.1), STAI (trait) levels and performance on the difficult mental-math task. If the data was not normally distributed then the Mann Whitney U non-parametric test was used to compare group differences. Version 19 of SPSS was used for data analysis. The strength of an association is reported as the squared Pearson product-moment correlation coefficient (r^2). A significance level of $P < 0.05$ was used to identify statistical significance.

RESULTS

Time to task failure and MVC data are reported in Chapter III. In brief, time to failure was less during the stress compared to the control session ($P < 0.05$) and similar for the mental-attentiveness and control session ($P > 0.05$). Maximal strength was greater for men than women ($P < 0.05$) but the reduction in strength for both were similar across sessions ($P > 0.05$). Men and women were similar in age, physical activity and trait anxiety levels ($P < 0.01$), but differed in height and weight ($P > 0.01$, Table 1, from Chapter III).

Errors during the mental-math (stressor) task at rest. The number of errors did not differ between men and women during the 4-minute mental math task (3.9 ± 1.3 vs.

3.6 ± 1.2, respectively, $P = 0.62$) nor during the fatiguing contraction (2.7 ± 0.9 vs. 2.6 ± 0.8, respectively, $P = 0.85$).

Perceived levels of anxiety. State anxiety (STAI) increased from baseline to after the cognitive stressor or quiet sitting (control) more for the stressor session (30.3 ± 7.3 to 39.6 ± 10.2) than the control session (33.2 ± 10.4 to 37.0 ± 11.2, session × time, $P = 0.04$). STAI was similar for both men and women (sex effect, $P = 0.53$) and with no sex difference across time (time × sex, $P = 0.69$). State anxiety increased from baseline to after the mental-attentiveness task (31.0 ± 7.9 to 34.4 ± 10.0) and after the quiet sitting (control session) (32.0 ± 9.9 to. 37.2 ± 11.7, time effect, $P = 0.006$) similarly (session × time, $P = 0.43$). There was no sex difference ($P = 0.66$) and no interactions ($P > 0.05$).

State anxiety increased after the stressor task more for the women that had elevated levels of trait (general) anxiety ($r = 0.71$, $r^2 = 0.50$, $P = 0.004$) but not for the men ($r = 0.43$, $r^2 = 0.18$, $P = 0.13$). Baseline levels of anxiety during the stressor session were associated with the error rate during the fatiguing contraction ($r = -0.54$ $r^2 = 0.29$, $P = 0.006$) indicating that those who had elevated levels of anxiety at baseline had less errors for the difficult mental-math task.

Cardiovascular responses during the cognitive stressor. Heart rate was greater during the 4 minutes of the mental math (stressor session) compared with the quiet sitting (control session) (81.9 ± 2.7 vs. 76.7 ± 2.5 $b \cdot min^{-1}$, respectively, session effect, $P = 0.04$, Figure 4.2A). There was no effect of sex ($P = 0.59$) and no interactions ($P > 0.05$).

MAP was also greater during the 4 minutes of mental math (stressor session) compared with the quiet sitting (control session) (101.3 ± 4.8 mmHg vs. 83.9 ± 0.8 mmHg, respectively, session effect, $P < 0.001$, Figure 4.2B). The increase in MAP was

the greatest during the first 30s and then remained elevated for the following 3.5 minutes (session × time, $P = 0.002$). MAP increased more for the men than the women for the stressor task (session × sex, $P = 0.02$).

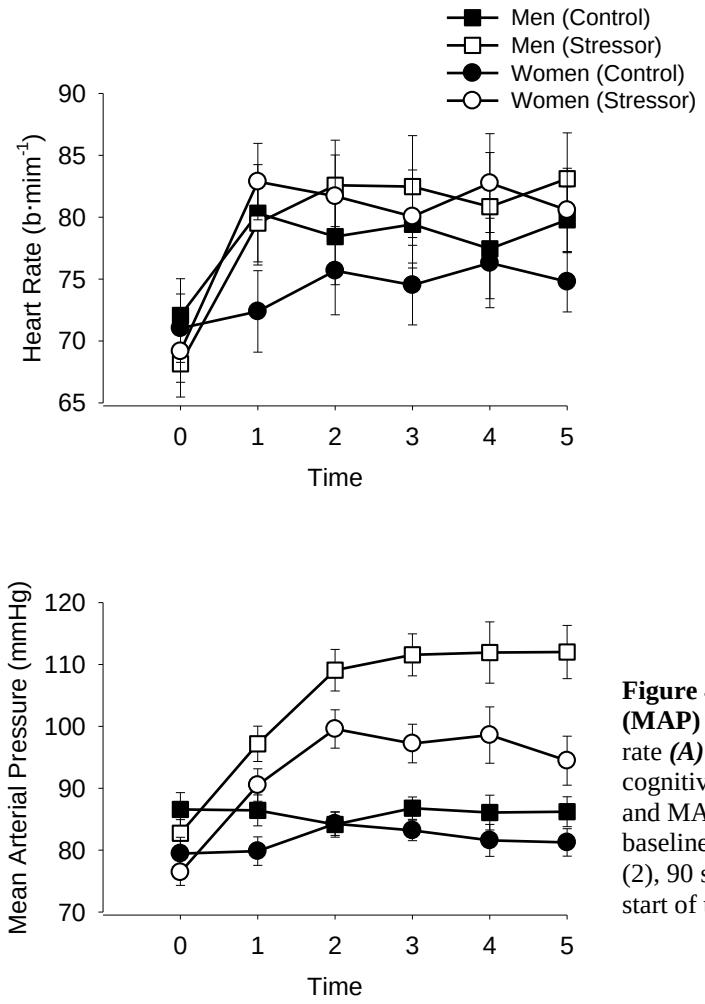

Figure 4.2. Heart Rate and Mean arterial Pressure (MAP) for Control and Stressor Sessions. Heart rate ***(A)*** and MAP ***(B)*** throughout exposure to the cognitive stressor or quite sitting (control). Heart rate and MAP were analyzed at 6 different time points; baseline (0), at the start of the first 2-min bout (1), 30 s (2), 90 s (3) and then at 30 s (4) and 90 s (5) after the start of the second 2 min of the cognitive task.

EMG during the cognitive stressor. EMG activity was measured during the cognitive tasks when muscles were expected to be resting. Neither biceps brachii nor the brachioradialis EMG increased (time effect, $P > 0.05$) during the cognitive stressor or quiet sitting (control) (session effect, $P > 0.05$) for men and women (sex effect, $P > 0.05$). Men tended to have slightly greater triceps activation throughout the cognitive stressor compared with the women (session × time × sex, $P = 0.06$).

Force fluctuations during the 5% contractions. Force fluctuations at baseline for the 5% steadiness task when averaged for all three sessions were greater for women compared with men (sex effect, $P = 0.01$). The amplitude of force fluctuations increased from baseline to after the cognitive stressor (session × time × sex, $P = 0.03$, Figure 4.4A) and after the mental-attentiveness task (session × time × sex, $P = 0.05$, Figure 4.5A) for the women but not for the men. Force fluctuations increased similarly after the fatiguing contraction (fatigue effect, $P < 0.001$) for men and women (fatigue × sex, $P = 0.21$) across sessions (session × fatigue × sex, $P > 0.05$) with no effect of sex ($P > 0.05$). During recovery, the force fluctuations remained elevated more for the women compared with the men (time × sex during recovery, $P < 0.001$) for the control and stressor session, but for not the control and mental-attentiveness session (time × sex during recovery, $P = 0.26$).

When men and women were pooled together, baseline CV for the 5% contraction was associated with average maximal strength (MVC) ($r = -0.53$, $r^2 = 0.29$, $P = 0.004$, Figure 4.3). The error rate during the 4 minutes of the difficult mental math (stressor session) was correlated with the force fluctuations for the 5% steadiness task immediately after the cognitive task for the women only ($r = 0.73$, $r^2 = 0.53$, $P = 0.04$) suggesting that women who had more errors were less steady.

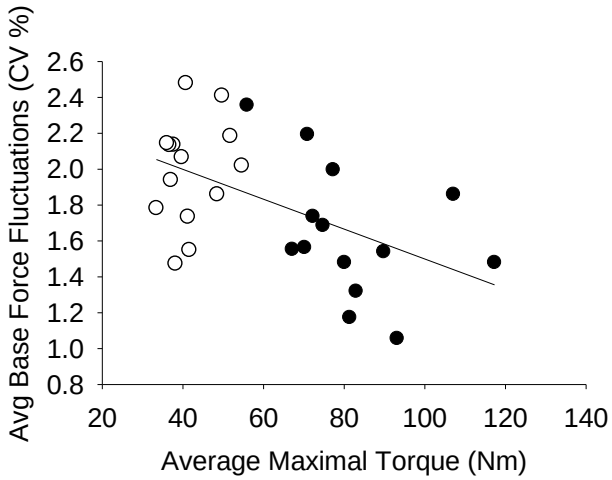

Figure 4.3. Association between Maximal Torque (Maximal Voluntary Contraction, MVC) and Force Fluctuations (CV). Association between baseline force fluctuations (CV) for the 5% contraction (averaged for all three sessions) and maximal torque (averaged for all three sessions) for men (closed symbols) and women (open symbols) (r = -0.53, r^2 = 0.29, P = 0.004).

EMG activity during the 5% contractions. Biceps brachii EMG activity during the 5% contraction was similar from baseline to after the cognitive stressor and quiet sitting (time effect, P = 0.10) but increased after the fatiguing contraction (fatigue effect, P < 0.001, Figure 4.4B) similarly for men and women (fatigue × sex, P = 0.25) for the control and stressor sessions (session × fatigue, P = 0.28). EMG activity remained elevated after fatigue (time effect during recovery, P = 0.91) with no effect of sex or session and no interactions (P > 0.05).

Biceps brachii EMG activity during the 5% contraction did not increase (time effect, P = 0.15, Figure 4.5B) after the mental-attentiveness task or quiet sitting (session × time, P = 0.30). Biceps brachii EMG increased after the fatiguing contraction (fatigue effect, P = 0.001) similarly for men and women (fatigue × sex, P = 0.29) for the control and mental-attentiveness sessions (session × fatigue, P = 0.29). EMG activity remained elevated after the fatiguing contraction (time effect during recovery, P = 0.37) with no effect of sex or session and no interactions (P > 0.05).

Brachioradialis EMG activity during the 5% contraction was similar from baseline to after the cognitive stressor and quiet sitting (time effect, $P = 0.47$, Figure 4.4C) with no differences between men and women (time × sex, $P = 0.96$) for the control and stressor sessions (time × session, $P = 0.73$). Brachioradialis EMG activity increased immediately after the fatiguing contraction (fatigue effect, $P = 0.03$) for both sessions similarly (session × fatigue, $P = 0.71$). EMG activity increased throughout recovery more for the women after the fatiguing contraction and exposure to the stressor (session × time × sex during recovery, $P = 0.01$).

Brachioradialis EMG activity during the 5% contraction did not increase (time effect, $P = 0.15$) after the mental-attentiveness or quiet sitting (session × time, $P = 0.26$, Figure 4.5C). Brachioradialis EMG increased after the fatiguing contraction (fatigue effect, $P = 0.03$) similarly for men and women (fatigue × sex, $P = 0.61$) for the control and mental-attentiveness sessions (session × fatigue, $P = 0.30$). EMG activity declined for the women after fatigue during the mental-attentiveness session compared with the men (session × time × sex during recovery, $P = 0.01$).

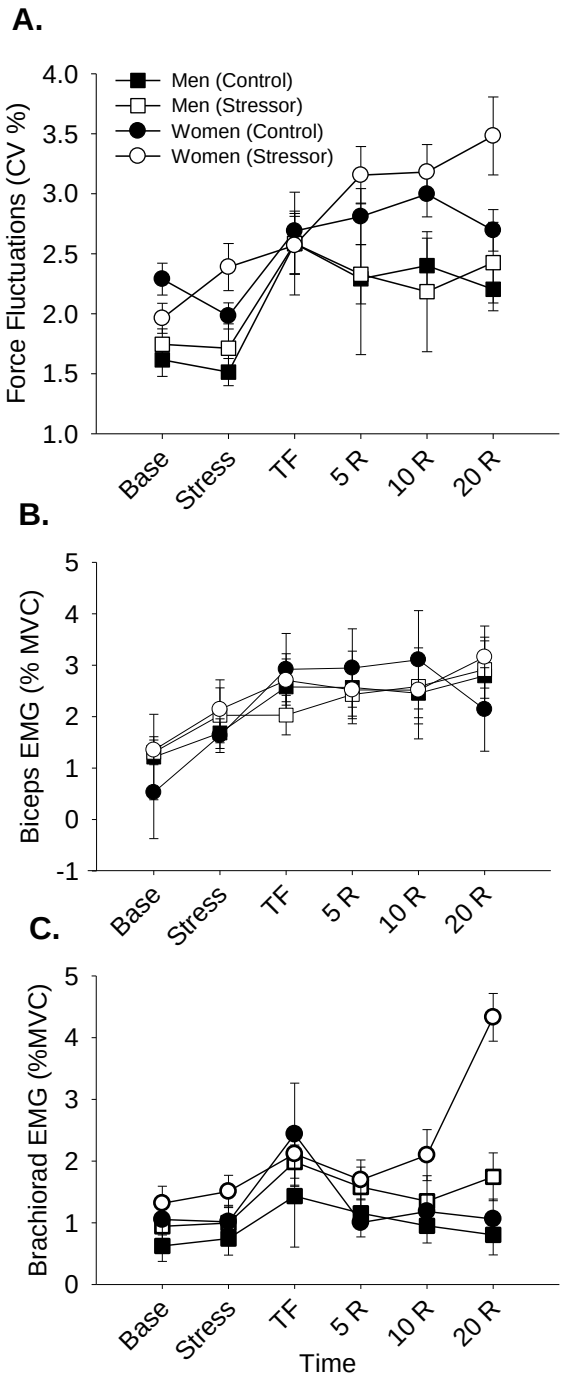

Figure 4.4. Force Fluctuations *(A)*, Biceps EMG *(B)* and Brachioradialis EMG *(C)* during the 5% (of MVC) Force Task for Control and Stressor Sessions. Force fluctuations increased for the women after exposed to the cognitive stressor ($P < 0.05$) ***(A).*** Force fluctuations increased after fatigue and throughout recovery ($P < 0.05$). Biceps EMG activity increased after the fatiguing contraction ($P < 0.05$) and remained elevated for men and women in both sessions ($P > 0.05$) ***(B)***. Brachioradialis EMG activity increased after the fatiguing contraction ($P < 0.05$) and continued to increase for women during the stressor session ($P < 0.05$) ***(A)*** Base: Baseline, Stress; immediately after the difficult mental-math or quiet sitting (control), TF: immediately after task failure, and 5, 10, 20 min after the fatiguing contraction (5 R, 10 R, 20 R).

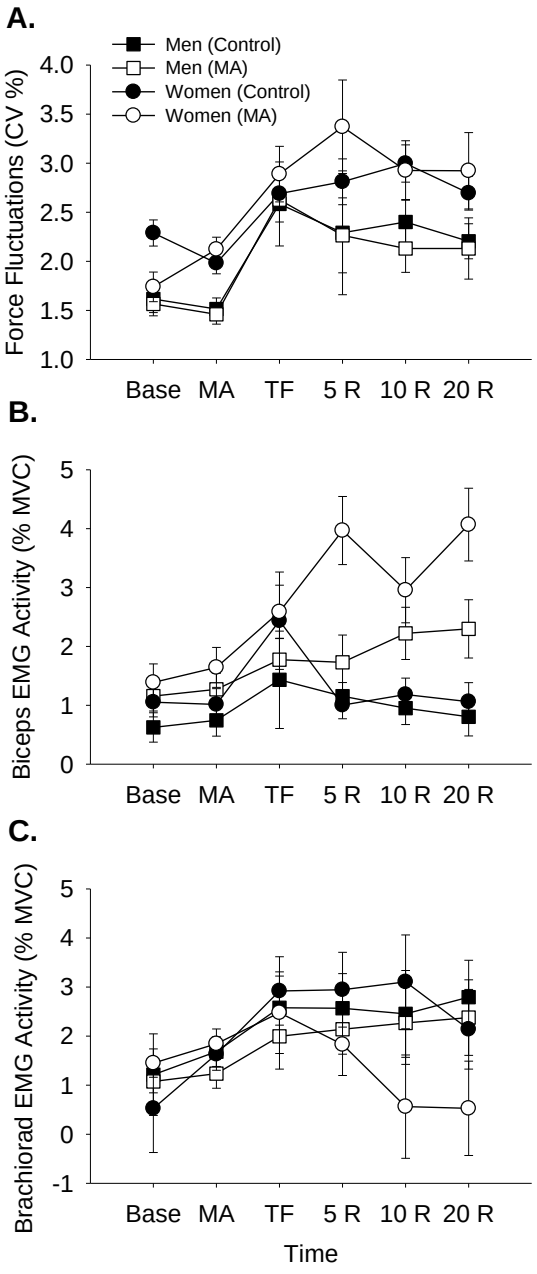

Figure 4.5. Force Fluctuations *(A)*, Biceps EMG *(B)* and Brachioradialis EMG *(C)* during the 5% (of MVC) Force Task for Control and Mental-attentiveness (MA) Sessions. Force fluctuations increased for the women after exposed to the mental-attentiveness task (MA) ($P < 0.05$) ***(A).*** Force fluctuations increased after fatigue and throughout recovery ($P < 0.05$). Biceps EMG activity increased after the fatiguing contraction ($P < 0.05$) and remained elevated for men and women in both sessions ($P > 0.05$) ***(B)***. Brachioradialis EMG activity increased after the fatiguing contraction ($P < 0.05$) and decreased for women during the MA session ($P < 0.05$) ***(C)*** Base: Baseline, MA; immediately after the simple mental-attentiveness tasks or quiet sitting (control), TF: immediately after task failure, and 5, 10, 20 min after the fatiguing contraction (5 R, 10 R, 20 R).

Cardiovascular responses during the 5% contraction. Heart rate, measured during the 5% contraction, increased from baseline to after the stressor and quiet sitting (control session) (time effect, $P = 0.01$, Figure 4.6A) similarly (session × time, $P = 0.68$) with no effect of sex ($P = 0.69$). Heart rate did not increase after the fatiguing contraction (fatigue effect, $P = 0.21$) but did increase throughout recovery (time effect throughout recovery, $P = 0.05$) similarly for both sessions and for men and women ($P > 0.05$). MAP

increased from baseline to after the stressor compared with the quiet sitting (session × time, $P = 0.007$, Figure 4.6B) with an overall effect of sex ($P = 0.005$) as men had greater MAP than women. MAP increased from baseline to after the fatiguing contraction (fatigue effect, $P = 0.001$) for both sessions, but MAP was greater overall for the men (sex effect, $P = 0.02$). MAP decreased throughout the recovery (time effect during recovery, $P = 0.003$) for men and women for both sessions ($P > 0.05$).

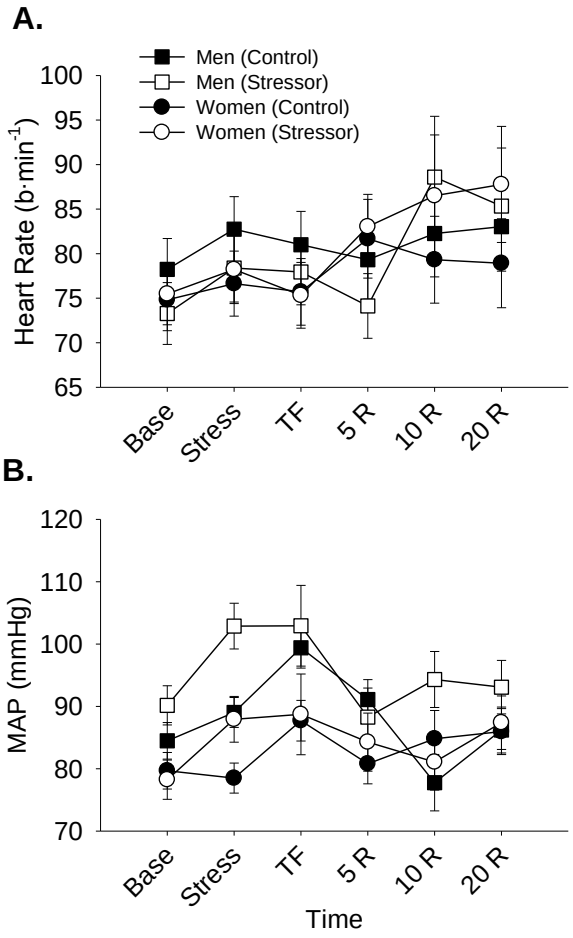

Figure 4.6. Heart Rate *(A)* and Mean Arterial Pressure (MAP *(B))* during the 5% (of MVC) Contraction for Control and Stressor Sessions. Heart rate increased after the stressor and quiet sitting ($P < 0.05$), but did not increase after the fatiguing contraction ($P > 0.05$). Heart rate increased throughout recovery ($P > 0.05$) ***(A)***. MAP increased after the stressor and fatiguing contraction ($P < 0.05$) for both sessions and decreased after the fatiguing contraction ($P < 0.05$) ***(B)***. Base: Baseline, Stress; immediately after the difficult mental-math or quiet sitting (control), TF: immediately after task failure, and 5, 10, 20 min after the fatiguing contraction (5 R, 10 R, 20 R).

Force fluctuations during the 20% fatiguing contraction: Force fluctuations increased throughout the 20% fatiguing contraction for both the control and stressor sessions (time effect, $P < 0.001$). The amplitude of force fluctuations was greater at the start of the contraction for the stressor session relative to the control for women compared

with men (session × time × sex, $P = 0.002$, Figure 4.7A). Rates of increase were different for men and women across sessions (session × sex, $P = 0.01$). Rates of increase for men were 0.57 ± 0.23 (control) and 0.62 ± 0.23 % CV·min^{-1} (stressor) and for women 0.41 ± 0.12 (control) and 0.23 ± 0.11 % CV·min^{-1}(stressor). Mental-attentiveness task did not affect the force fluctuations during the 20% contraction (Figure 4.8A, $P > 0.05$).

Anxiety (STAI) after the mental math was associated with the force fluctuations at the start ($r = 0.56$, $r^2 = 0.31$, $P = 0.04$) and the end ($r = 0.57$, $r^2 = 0.33$, $P = 0.04$) of the fatiguing contraction for the women only. Maximal strength was not associated with force fluctuations at the start of the 20% contraction ($r = -0.04$, $r^2 = 0.002$, $P = 0.84$).

EMG activity during the fatiguing contraction. Biceps brachii EMG increased throughout the fatiguing contraction (time effect, $P < 0.001$) similarly for both the control and stressor session (session × time interaction, $P = 0.95$, Figure 4.7B) with no effect of sex ($P = 0.92$). Brachioradialis EMG increased during the fatiguing contraction (time effect, $P < 0.001$) similarly for the stressor compared with the control session (session × time interaction, $P = 0.24$, Figure 4.7C) with a trend for men and women to have different EMG patterns during the stressor session compared with the control (session × time × sex, $P = 0.06$), where men tended to have less brachioradialis EMG during the stressor session, women tended to have greater brachioradialis EMG levels during the stressor session.

Triceps EMG increased (time effect, $P < 0.001$) similarly for men and women throughout the fatiguing contraction (time × sex, $P = 0.93$) for the stressor session compared with the control session (session × time × sex, $P = 0.12$) with no main effect of session (session effect, $P = 0.75$). Coactivation of the agonist and antagonist muscles

(biceps and triceps, respectively) was similar throughout the fatiguing contraction (time effect, $P = 0.82$) for control and stressor session (session effect, $P = 0.24$) for men and women (sex effect, $P = 0.46$) with no interactions ($P > 0.05$). Coactivation levels for men were 49.1 ± 25.4 % for the control session and 67.6 ± 44.0 % for the stressor session and for women were 48.9 ± 30.1 % for the control session and 48.9 ± 30.1 % for the stressors session.

Biceps brachii and triceps EMG increased throughout the fatiguing contraction (time effect, $P < 0.05$) for both the mental-attentiveness and control sessions (session × time, $P > 0.05$, Figure 4.8B (Biceps only)). There was no effect of sex ($P > 0.88$) and no significant interactions ($P > 0.05$) for force fluctuations, biceps brachii or triceps EMG between the control and mental-attentiveness sessions.

Brachioradialis EMG increased throughout the fatiguing contraction differently for men and women throughout the control and mental-attentiveness session (session × time × sex, $P = 0.03$, Figure 4.8C). Women had greater increases in their EMG levels during the mental-attentiveness task relative to their control session compared with the men.

There was a progressive increase in the number of bursts of EMG in the biceps brachii muscle throughout the fatiguing contraction for both tasks (time effect, $P < 0.001$). Overall, the bursting activity was similar for the stressor and control session (session effect, $P = 0.08$) but increased at a lower rate for the stressor session (session × time, $P = 0.03$, Figure 4.7D). There were no sex differences in the bursting activity (sex effect, $P = 0.08$). The mental-attentiveness task did not affect bursting activity throughout the fatiguing contraction (session effect, $P = 0.55$, Figure 4.8D), there were no sex

differences ($P = 0.13$) and no interactions ($P > 0.05$) for bursting activity throughout the fatiguing contraction for the control and mental-attentiveness session.

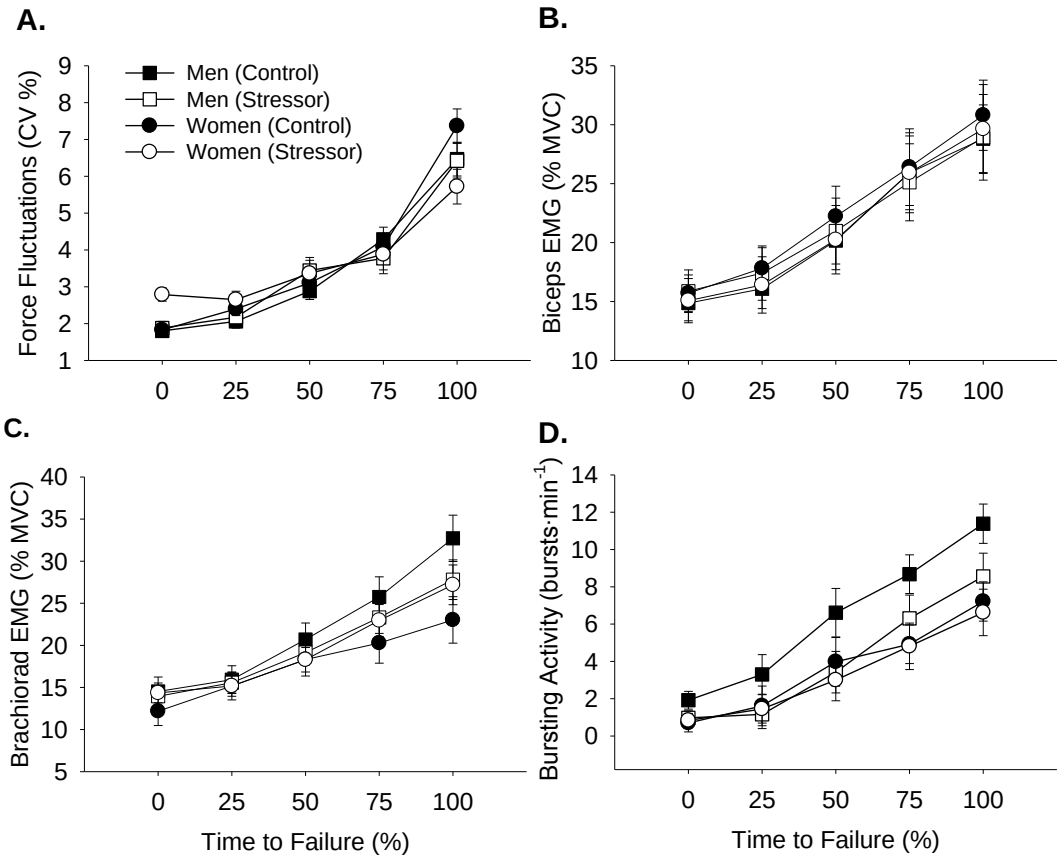

Figure 4.7. Force Fluctuations, Root Mean Squared (RMS) EMG Activity for the Biceps Brachii and Brachioradialis and Bursting EMG for Biceps Brachii Pooled during the Fatiguing Contraction. Force Fluctuations for the 20% fatiguing contraction *(A)*. RMS EMG of biceps brachii (*B*) and brachioradialis (*C)* normalized to the MVC values (% MVC) during the fatiguing contraction and bursting activity of the biceps brachii (*D)*. Shown is the mean (± SEM) of 15 s intervals in 25% increments of the time to task failure.

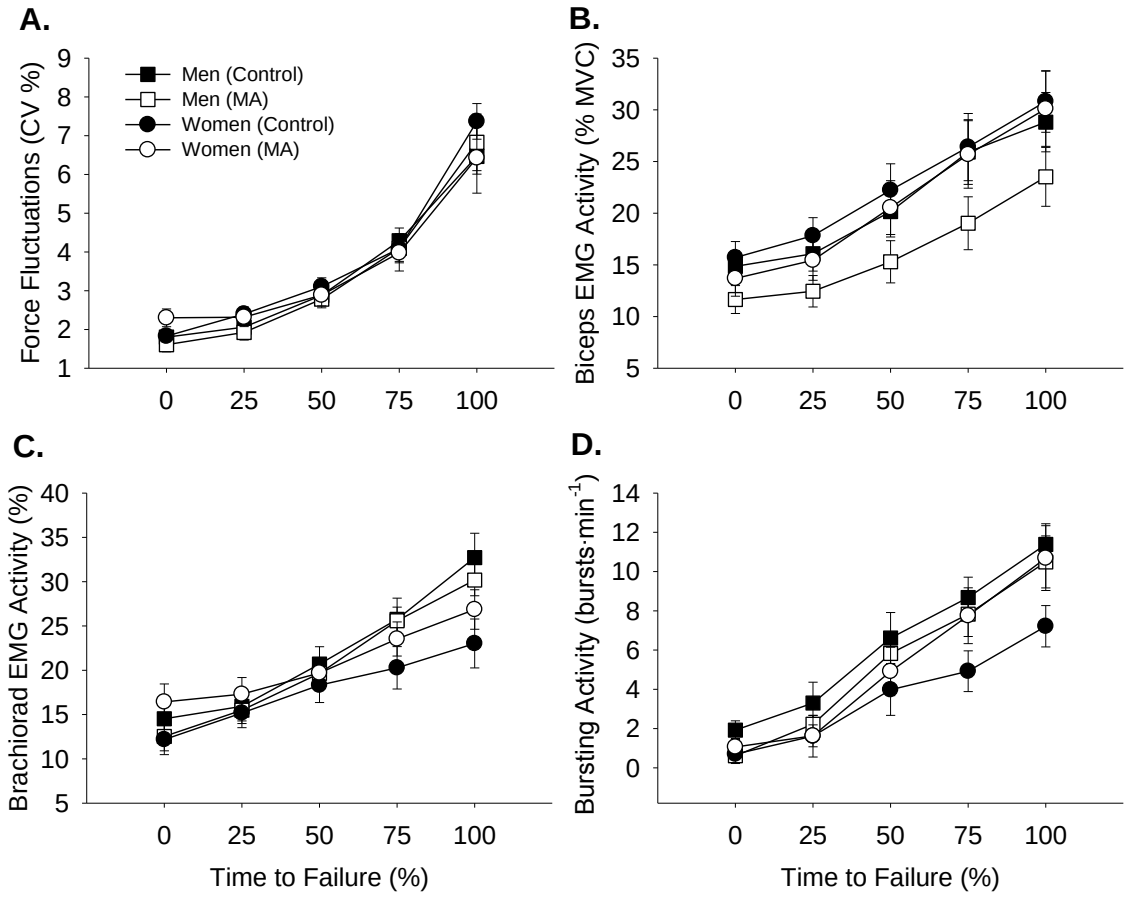

Figure 4.8. Force Fluctuations, Root Mean Squared (RMS) EMG Activity for the Biceps Brachii and Brachioradialis and Bursting EMG for Biceps Brachii Pooled during the Fatiguing Contraction for the Control and Mental Attentiveness (MA) session. Force Fluctuations for the 20% fatiguing contraction ***(A)***. RMS EMG of biceps brachii **(*B*)** and brachioradialis ***(C)*** normalized to the MVC values (% MVC) during the fatiguing contraction and bursting activity of the biceps brachii ***(D)***. Shown is the mean (± SEM) of 15 s intervals in 25% increments of the time to task failure.

DISCUSSION

The purpose of this study was to determine if there was a sex difference in force fluctuations for a very low-intensity contraction (5% of MVC) with the elbow flexor muscles before and after a fatiguing contraction in the presence and absence of a cognitive stressor. The main findings from this study were that 1) women but not men had an increase in force fluctuations for the 5 % contraction after the cognitive stressor and the mental-attentiveness tasks, prior to the fatiguing contraction, 2) both men and

women had increases in force fluctuations for the 5 % contraction for all three sessions immediately after the fatiguing contraction, but the sex differences disappeared and 3) force fluctuations remained elevated throughout 20 minutes of recovery for women compared with the men for the control and stressor session. An additional finding of this study was that force fluctuations for the 20% contraction were more elevated at the start of the contraction for women during the cognitive stressor, but that coactivation was not different during the 20% fatiguing contraction and therefore, did not contribute to differences in force fluctuations or time to failure in the presence of a stressor.

Sex Differences in Force Fluctuations in the Absence of Stress

This study demonstrated that in the absence of stress women were less steady than men for the 5% task. At the start of the 20% contraction for the control condition, force fluctuations were similar between men and women. This is consistent with previous studies of the elbow flexor muscles (Brown et al., 2010). Maximal strength (baselines averaged across sessions) was associated with force fluctuations at 5% of MVC (baseline CV averaged across sessions) but not 20% of MVC during the control condition, suggesting at least for the 5% contraction, individuals who were stronger (mainly men) were more steady than the weaker individuals, shown previously (Brown et al., 2010).

The amplitude of force fluctuations is modulated by motor unit recruitment and discharge frequency (Taylor et al., 2003). For low-intensity contractions, mainly Type I motor units are recruited. Women usually have smaller muscles and possess a greater proportion of Type I fibers (Simoneau & Bouchard, 1989; Roepstorff et al., 2006). Additionally, discharge rates were shown to be lower in women when the cognitive load was increased during a 20% fatiguing contraction with the elbow flexor muscles

(Mottram et al., 2006). Discharge rate variability generally increases as the discharge rate decreases (Taylor et al., 2003). This would suggest that the discharge rate variability may be greater in women under stressful conditions. Therefore, the greater force fluctuations for the low-intensity contractions may be due to less absolute force generated by women to sustain the 5% task and a greater discharge rate variability in women compared with men.

Sex Differences in Motor Output Variability are Exacerbated with Stress

Exposure to the cognitive stressor (prior to the fatiguing contraction) increased MAP and heart rate for both men and women compared with quiet sitting indicating that the stressful cognitive task likely increased sympathetic activation. Men and women also had similar cognitive performance for the difficult mental-math tasks quantified by the error rates per minute. Anxiety levels were similar in men and women and those with elevated anxiety performed better on the mental-math task (cognitive stressor). Women, but not men, with elevated trait anxiety levels had greater state anxiety after exposure to the cognitive stressor and women who had greater baseline anxiety levels for the stressor session had greater force fluctuations for the start and end of the 20% fatiguing task during the stressor session. Force fluctuations were overall greater for women at the start of the 20% contraction during the stressor session and for the 5% contraction after exposure to both the cognitive stressor and mental-attentiveness task.

Increases in sympathetic activation from stress may enhance monoaminergic drive to the motoneuron pool which can augment the gain between the input received by the motoneuron pool and its output (Heckman et al., 2009). Alternatively, there is evidence for depressed muscle spindle (afferent feedback to the motoneuron) discharge

with an activation of the sympathetic nerve (Roatta et al., 2002; Hellstrom et al., 2005) which may be due to norepinephrine mediated calcium influx inhibition (Marchetti et al., 1986; Cox & Dunlap, 1992; Dolphin, 1995). It is possible for both the depressed spindle activity to the motoneuron and augmented input-output gain of the motoneuron pool to alter force fluctuations. Heart rate, mean arterial pressure and EMG were similar for men and women for the 5% contractions before and after exposure to the cognitive stressor, indicating that indices of neural and sympathetic activation after the stressor alone may have been similar for men and women. Additionally, because force fluctuations increased after both the cognitive stressor and mental-attentiveness task, it is possible that the women had greater force fluctuations after the fatiguing contraction for the 5% contraction because of greater difficulty in concentration.

Sex Differences in Motor Output Variability throughout Recovery

The sex difference in force fluctuations no longer existed at the 5% contraction force immediately after the fatiguing contraction. Muscle fatigue can alter force fluctuations for a target matching task, by altered Ia afferent input to the alpha motoneuron during a sustained contraction (Macefield et al., 1991; Cresswell & Loscher, 2000), increase in reflex inhibition from group III and IV muscle afferents (Woods et al., 1987) increased synchronization of motor units (Holtermann et al., 2009) (which is greater among motor units with smaller and slower twitches) (Schmied et al., 1993; Schmied et al., 1994) and coactivation of the antagonist (Vallbo & Wessberg, 1993; Spiegel et al., 1996). It is possible that immediate changes that occur at the motoneuron with fatigue, i.e. feedback from the reflex afferents to the motoneuron) may be modulated

similarly for the very low-intensity contraction in men and women and this may override causative factors that increased force fluctuations with greater cognitive loads.

During recovery however, the sex difference in force fluctuations at the 5% contraction returned and women had elevated force fluctuations throughout recover. This can be considered either a delayed response or slower recovery in the women. Regardless, this may suggest greater long-term effects of fatigue on feedback to the motoneurons and input-output activity of motor units, causing greater muscle tremor in women during low-intensity contractions during recovery from fatigue. Heart rate continued to be elevated indicating greater sympathetic activity (from the fatiguing contraction) or greater parasympathetic withdrawal throughout recovery but this was similar for men and women. Brachioradialis EMG was significantly greater at 20 min for the women after the stress and fatigue for the stressor session and may have contributed to the increased force fluctuations for women at 20 minutes of recovery.

Force Fluctuations during the 20% Fatiguing Contraction

Force fluctuations increased during the 20% contraction, more for men than women likely because they reached task failure more quickly. Women had greater force fluctuations at the start of the 20% contraction for the stressor session but the rate of rise was slower compared with the control session. Coactivation during the fatiguing contraction in this study was similar for both sessions in men and women, suggesting that coactivation of the agonist and antagonist muscles did not contribute to differences in force fluctuations or the differences in time to failure for the control and stressor session. Increases in surface EMG during the fatiguing contraction were similar for both the stressor and control session for the biceps brachii, brachioradialis and the triceps muscles.

Women tended to have greater increases in the brachioradialis with exposure to the stressor and the mental attentiveness-task, suggesting differential muscle activation patterns during a fatiguing contraction with increased cognitive load.

Bursting EMG activity, a representation of the transient recruitment of motor units (Kouzaki et al., 2002), was less during the stressor session compared to the control session. It might be expected that the increase in sympathetic activity from the stress may alter intrinsic properties of the muscle by transient potentiation in Type II motor units (Bowman, 1980). Nevertheless, it appears that there is less transient activity for the fatiguing contraction when exposed to the stress compared with the control session and this may be caused by a reduced afferent feedback to the motoneuron pool during the stressor task (Roatta et al., 2002).

In conclusion, women are less steady than men for very low-intensity contractions in the presence and absence of a cognitive stressor and a mental-attentiveness task for the elbow flexor muscles. The sex differences are abolished immediately after the fatiguing contraction and then return as women have a slower of force fluctuations after a fatiguing motor task compared with the men. The mechanisms for greater force fluctuations in women for the very low-intensity contraction at baseline, although not completely clear, may be due to less absolute strength and greater proportion of Type I fibers. In this study it does not appear that the greater fluctuations in force after exposure to stress were due to greater sympathetic activity in the women but may be due a lesser ability to concentrate after the cognitive tasks. Furthermore, fatigue may contribute to more long-term deficits in motor performance during target matching tasks that require fine motor control and precise movements in women compared with men.

Chapter V

Muscle Fatigability is Greater in Veterans with Posttraumatic Stress Disorder

SUMMARY

This study determined the fatigability and steadiness in the hand muscles of veterans with post-traumatic stress disorder (PTSD) in the presence and the absence of an acute stressor. Thirty-nine men (20 veterans with PTSD, 36 ± 9 years, and 19 controls, 28 ± 9 years) participated in a control session and then subgroups of these subjects participated in additional sessions. The study involved three experimental sessions (control, stressor and mental-attentiveness) and an additional pain session. Each session (except the pain session) involved an isometric fatiguing contraction (20% of maximal strength) with the handgrip muscles. The stressor session included a difficult mental-math task and the mental-attentiveness session a simple mental-math task, before and during the fatiguing contraction. In a separate session for a subset of subjects, pain was assessed by a pain/pressure device that was placed on the index finger of the right hand for 2 min and by a McGill questionnaire which documented symptoms of chronic pain. Baseline levels of anxiety, heart rate and mean arterial pressure were greater for veterans with PTSD ($P < 0.05$). Steadiness was reduced ($P < 0.05$) and time to failure was briefer for veterans with PTSD compared with control subjects (7.2 ± 2.5 vs. 9.3 ± 5.2 min, respectively, $P = 0.03$). Mean arterial pressure and the rate pressure product were elevated more for veterans with PTSD compared with controls throughout the fatiguing

contraction ($P < 0.05$). Time to failure was less for veterans for both control and stressor sessions ($P < 0.05$) but the acute cognitive stressor did not alter time to failure or force fluctuations for veterans with PTSD or control subjects ($P > 0.05$). Pain thresholds were lower in veterans with PTSD and symptoms and intensity of pain (McGill) were greater in veterans with PTSD ($P < 0.05$). Symptoms of pain (McGill) correlated with time to failure ($r = -0.61$, $r^2 = 0.37$, $P = 0.007$). The results of this study demonstrate important clinical implications for motor performance in vocational and military settings for veterans with PTSD.

INTRODUCTION

Physiological responses to acute stress are important determinants of health because they can increase vulnerability to stress/anxiety disorders and musculoskeletal disorders (Holden, 2005; Passatore & Roatta, 2006). The physiological adaptations that occur from an acute traumatic stressor can have detrimental and long lasting psychological effects leading to posttraumatic stress disorder (PTSD). PTSD can be caused by the threat of death or serious injury that leads to a reaction of intense fear, helplessness or horror (APA, 2000). PTSD involves a) re-experiencing the traumatic event, b) avoidance of stimuli and emotional numbing and c) symptoms of hyperarousal (APA, 2000). Veterans of war often have a high prevalence of combat or military related PTSD with a lifetime prevalence of PTSD of 15% in Iraq and Afghanistan war veterans (Schnurr, 2010). The physiological adaptations that are known to occur with PTSD are a dysregulation in the stress systems [hypothalamic pituitary adrenal (HPA) axis and sympathetic nervous system] (McFall et al., 1992; Olff et al., 2006). For example, people with PTSD demonstrate elevated levels of sympathetic activation (increased plasma

epinephrine, norepinephrine and serotonin) (Southwick et al., 1999) and higher resting levels of heart rate and mean arterial pressure (Bedi & Arora, 2007).

Sympathetic activation is well known to support motor function including modulating blood perfusion (Seals, 2006) to the muscle and monaminergic drive to the motoneurons (Heckman et al., 2009). Though in the presence of stress, sympathetic outflow will result in excessive and inappropriate actions on motor control (Roatta et al., 2002; Passatore & Roatta, 2006; Roatta et al., 2008). Sympathetic activation exerts a number of actions at the periphery, including modulation of skeletal muscle contractility (Bowman, 1980), altered blood perfusion to skeletal muscles (Joyner & Dietz, 2003; Thomas & Segal, 2004), and modulation of the discharge of numerous receptors (i.e. muscle spindles, which carry afferent feedback to the muscle for adequate motor control) (Roatta et al., 2002; Hellstrom et al., 2005). Furthermore, increased sympathetic activation will upregulate neuromodulators that can augment the synaptic activity at the spinal cord level (Heckman et al., 2003) and may alter motor output, although whether this will impair or enhance motor performance is unknown. A chronic increase in sympathetic activity therefore in people with PTSD may influence muscle fatigability and steadiness during low-intensity contractions.

In healthy young adults, an acute stressor can increase force fluctuations and muscle fatigability during low-intensity isometric contractions (Noteboom et al, 2001b; Christou et al., 2004; Yoon et al., 2009; Chapter III). Motor performance that requires low-intensity tasks during daily activities, have not been examined in patients with PTSD. The only known motor performance-related study in veterans with PTSD assessed reaction time. Reaction time to an auditory task was reported to be greater in individuals

with PTSD (McFarlane et al., 1993) while other studies showed no differences in reaction time compared with healthy controls (Metzger et al., 1997; Galletly et al., 2001). The impairment in reaction time was suggested to be related to a dysfunction in the regulation of norepinephrine (McFarlane et al., 1993). Other motor tasks however, such as sustained low-intensity contractions that are the foundation of stabilizing tasks performed in vocational and military settings, are potentially impacted in veterans with PTSD but have not been investigated. The first aim of this study therefore, was to determine if veterans with PTSD fatigued more quickly and were less steady than control subjects for a low-intensity, target matching contraction with the handgrip muscles. Because people with PTSD have elevated basal levels of sympathetic activation (measured by plasma neuromodulators) (Southwick et al., 1999) which may influence motor performance, we hypothesized that veterans with PTSD would fatigue more quickly and be less steady than control subjects.

Furthermore, most vocational and military tasks are executed while performing a cognitive task or under stressful conditions. Individuals with PTSD have greater physiological responses to acute stressors than healthy adults including elevated heart rates and blood pressure and increased skin conductance (measures of the sympathetic nervous system activity) when exposed to a stressor (Pitman et al., 1987; Pitman et al., 1990; Orr et al., 1993). It is unknown if the greater stress response demonstrated in individuals with PTSD will affect fatigability of the muscle or the ability to maintain a steady contraction. Therefore, the second aim of this study was to determine if exposure to an acute cognitive stressor will cause greater impairments in motor performance (increase muscle fatigability and reductions in steadiness) for low-intensity contractions

of the handgrip muscles in veterans with PTSD. We hypothesized that fatigability and steadiness would be even more impaired for veterans with PTSD when exposed to the acute stressor.

The frequency and intensity of exposure to combat experiences is strongly associated with the risk of chronic PTSD (APA, 2000) and related cognitive and physical impairment (Kaylor et al., 1987; Kessler, 2000; Dunn et al., 2011). In addition, individuals with PTSD have significant reductions in physical activity levels (de Assis et al., 2008) and report greater symptoms of musculoskeletal disorders and pain (Dunn et al., 2011). Therefore, physical activity levels and acute and chronic perceptions of pain were assessed to determine if they were associated with muscle fatigability for low-intensity motor tasks.

METHODS

Study Overview

A total of 39 subjects participated in this study to investigate two aims. Twenty male veterans with PTSD and 19 male civilian control subjects participated in a session to perform a fatiguing contraction with their handgrip muscles at 20% of the maximal voluntary contraction (MVC) (Aim 1). Twenty male veterans with PTSD and 12 male control subjects attended an additional session to perform a difficult mental-math task simultaneously during the fatiguing contraction (Aim 2). We have previously demonstrated that fatigability is not greater because of the distraction from performing a cognitive task simultaneously during the fatiguing contraction in healthy young adults (Yoon et al., 2009). To determine this in veterans with PTSD, 20 male veterans with PTSD and 6 control subjects attended a third session where they performed a simple

mental-math task during the fatiguing contraction (mental-attentiveness session). Finally, a subset (9 male veterans and 6 male civilian controls) attended a third session to determine pain perceptions with the McGill pain questionnaire and pressure/pain device. Veterans with PTSD were diagnosed using the Diagnostic and Statistical Manual IV (DSM IV) by a physician and were Operation Enduring Freedom (OEF) or Operation Iraq Freedom (OIF) combat veterans with PTSD related to combat experiences. The medications for the PTSD group were not controlled but were documented during the initial session.

All testing occurred at the Veteran Affairs Medical Center (VAMC) for the veterans and Marquette University for the control subjects. The equipment was similar at each site. The protocol was approved by the institutional review board at the VAMC and Marquette University. Prior to participation in the study, each subject provided informed consent. At the initial familiarization session, all subjects completed health questionnaires and were familiarized to the equipment and practiced some experimental procedures. Because substance abuse can often be comorbid with PTSD (Kozaric-Kovacic, 2009), veterans provided a urine sample to test for illicit drug use. Subjects also answered questionnaires regarding their general anxiety levels (State Trait Anxiety Inventory, STAI-Trait) (Spielberger, 1970), symptoms of PTSD using the PTSD Checklist-Civilian (PCL-C) (Wilkins et al., 2011) and symptoms of depression with the Beck Depression Inventory (BDI) (n = 31, only 10 control subjects filled out the PCLC and BDI) (Beck et al., 1961). Body mass index (BMI) was calculated from height and weight measurements and physical activity levels for each subject were assessed with a questionnaire that estimated the relative kilocalorie expenditure per week. Participants then practiced

maximal voluntary contractions (MVCs) and brief submaximal target matching contractions at 20% of MVC force with the left hand (except one veteran in which the right hand was tested because he had previously broken the left wrist). Subjects were instructed to abstain from caffeine, exercise and smoking (7 veterans smoked cigarettes) on the days of testing and alcohol 24 hours prior to testing. All control subjects were healthy with no known neurological or cardiovascular diseases and were naive to the protocol. Control subjects had low-to-moderate levels of general anxiety (32 ± 7) quantified with the trait score of the STAI (Spielberger, 1970).

Experimental Protocol for Control, Stressor and Mental-attentiveness Session

Each experimental session began with assessments of baseline levels of anxiety using the visual analogue scale (VAS) (Johnson, 2001) and STAI (state levels). All procedures were performed in the following order for each experimental session (Figure 5.1): (1) MVCs of the handgrip muscles, (2) assessment of cognitive and physiological arousal before and after either quiet sitting (control session) or 4 minutes (2 x 2-min bouts) of mental tasks (stressor and mental-attentiveness sessions only), (3) performance of a fatiguing contraction at 20% MVC force with the handgrip muscles and (4) recovery MVCs and assessments of cognitive and physiological arousal immediately after the fatiguing contraction and at 2, 5 and 10 min recovery.

Pre-fatigue measures. Two to three MVCs were performed at the beginning of each session. The peak MVC was used to calculate the required force for the fatiguing contraction at 20% MVC. Electromyography (EMG) of the finger flexors and extensors were recorded during the MVC and used to normalize the EMG during the fatiguing contraction.

Fatiguing contraction. A fatiguing contraction was performed with the handgrip muscles at 20% MVC force during each experimental session. The subject was required to match the vertical target force as displayed on the monitor and was verbally encouraged to sustain the force for as long as possible. To minimize the influence of transient fluctuations in motor output on the criteria for task failure, the task was terminated only after force fell below 10% of the 20% contraction for 2 out of a 4-s interval.

The following variables were recorded during the fatiguing contraction; heart rate, blood pressure, finger flexor and extensor EMG and rate of perceived exertion (RPE) as an index of perceived effort (Borg, 1982). For RPE, each subject was instructed to focus the assessment of effort on the arm muscles performing the fatiguing task. The RPE scale was anchored so that 0 represented the resting state and 10 corresponded to the strongest contraction that the upper limb muscles could perform. RPE was recorded at the beginning of the fatiguing contraction and every minute thereafter until task failure.

Recovery measures. Recovery measures of MVCs and VAS for anxiety were assessed at the following times: immediately upon task failure, 2, 5, and 10 min after termination of the fatiguing contraction (Figure 5.1).

Cognitive Tasks

Mental math is a well-established psychosocial technique to induce stress (Noteboom et al., 2001b; Kajantie & Phillips, 2006) and was used to increase levels of anxiety and stress in the earlier studies (Chapters II and III) and our other studies (Yoon et al., 2009). During the stressor session, each subject performed serial subtraction from a 4-digit number by 13 with a response required every 3 s. Once the subject made an error

in the math or was not able to provide the correct answer within 3 s, they were asked to start the mental math again from a new number in the series. Each subject performed the mental math during the stressor session only. They performed the mental math before the fatiguing contraction (2 x 2 min bouts) and then continuously during the fatiguing contraction.

The mental-attentiveness task required subjects to perform a simple math task that was not designed to induce stress as we had done in young healthy subjects (Yoon et al., 2009) (also in Chapters II and III). Participants subtracted by one from 50 continuously during the 4 minutes (2 x 2 min bouts) while at rest prior to the fatiguing contraction and during the fatiguing contraction in the mental-attentiveness session.

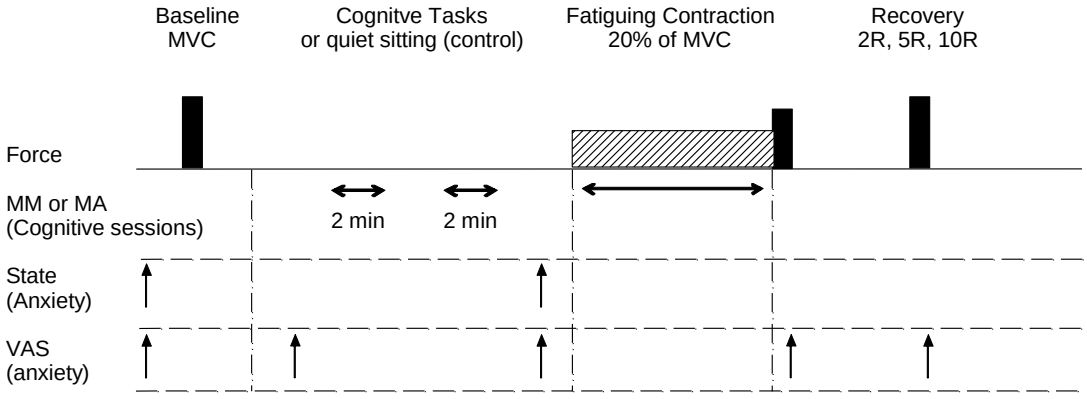

Figure 5.1. Experimental Protocol. The top panel shows the order of force tasks performed by each subject with the handgrip muscles. Three MVCs were performed. This was followed by a fatiguing contraction at 20% of MVC and recovery MVCs (2R, 5R and 10R). Mental math (MM), mental attentiveness (MA) or quiet rest (control session) were performed 2 x 2 min (total of 4 minutes) before and then during the fatiguing contraction for each respective session 2nd row). State portion of the State-Trait Anxiety Inventory (STAI) questionnaire was assessed twice throughout the protocol. Levels of anxiety using the visual analogue scale (VAS) were assessed throughout the protocol. Note that the schematic is not to scale for time or force.

Mechanical Recordings

Each subject was seated upright in an adjustable chair with the left arm slightly abducted and the elbow, forearm and wrist resting on a padded support at or slightly

above heart level. The elbow joint was flexed to 90 degrees so that the forearm was horizontal to the ground and the wrist midway between supination and pronation. The motor task involved gripping a custom-made adjustable handgrip dynamometer. The tensile force detected by the transducer was recorded on-line at 1000 samples/s using Biopac Pro software (*Biopac* Systems Inc., Goleta CA) and displayed on a 15-inch monitor 1.0 m in front of the subject. Each subject was asked to trace the horizontal force signal for as long as possible during the fatiguing contraction. The force signal appeared on the screen from the left side of the monitor at 2 cm/s.

Electrical Recordings

EMG signals were recorded with bipolar surface disposable electrodes (Ag-AgCl, 8-mm diameter; 16 mm between electrodes) that were placed over the flexor digitorum and extensor digitorum muscles. The bipolar electrode configuration was placed longitudinally over the muscle belly midway between the origin and insertion for each muscle, according to the European recommendations for surface electromyography (Hermens et al., 2000). Reference electrodes were placed on a bony prominence at the elbow. The EMG signal was amplified (1000×), band-pass filtered (30-1000 Hz) and sampled at 1000 samples sec^{-1} with Biopac systems.

Cardiovascular Measurements

Heart rate and blood pressure were monitored at rest (baseline) and also during the fatiguing contraction. Heart rate was recorded from a heart rate monitor (Polar F1 Heart Rate Monitor, Oulu, Finland) placed against the skin around the subjects chest wall at heart level. Blood pressure was monitored with an automated wrist cuff (HEM-670IT, OMRON Electronic Components, Schaumberg, Illinois). The blood pressure cuff was

placed around the wrist of the right hand, with the hand placed on a table adjacent to the subject at heart level. The automated blood pressure signal was calibrated to a manual blood pressure at each session. The blood pressure and heart rate was monitored and documented at the start of the contraction and every minute thereafter until task failure.

Cognitive Assessment of Arousal

Cognitive levels of anxiety were assessed throughout the protocol using VAS and the state portion of the STAI questionnaire as we have detailed previously (Yoon et al., 2009; Keller et al., 2011). In brief, the VAS involved a 10-cm line anchored at the far left by "not at all anxious" and at the far right by "very anxious." Anxiety was defined as the emotional changes perceived by the subject which was above and beyond the expectation for their level of exertion (Christou et al., 2004). The subject indicated their level of anxiety on the horizontal line of the scale. VAS for anxiety were recorded at 7 time points during the protocol: two baseline assessments; immediately after the cognitive task or quiet sitting and prior to the start of the fatiguing contraction, immediately after the fatiguing contraction; and then 2, 5, 10 min after fatiguing contraction (Figure 5.1).

The STAI-state questionnaire involved 20 statements that required a response on a four-point, Likert-type scale. Assessment of STAI was performed at baseline and prior to the fatiguing contraction (Figure 5.1).

Assessments of Pain Perception

Pain perception was assessed with a McGill pain questionnaire and with a pain/pressure device (Hoeger Bement et al., 2008). The McGill questionnaire (Melzack, 1975) obtained subjective measures of chronic pain. The McGill questionnaire is a 3-

dimentional pain assessment tool that quantifies pain in sensory, affective and evaluative components. Measures of the McGill pain questionnaire include the pain rating inventory and the present pain intensity.

Pain/pressure assessment: To assess pain a 200 g mass was applied to a second class lever a distance from the axis to ensure a 10 N force (equivalent to a 1 kg mass) on the index finger of the right hand (Hoeger Bement et al., 2008). The force at the finger was applied through a Lucite edge (8 x 1.5 mm) (Romus Inc., Milwaukee, WI, USA) and was placed on the right index finger midway between the distal and proximal interphalangeal joints for 2 min. Subjects were instructed to say *pain* when they first felt pain (i.e., pain threshold) and pain ratings were reported every 20 s using a 0-10 scale. The scale had the following terminology: 0 = no pain, 5 = moderate pain, and 10 = worst pain (McCaffery & Pasero, 1999). This procedure results in a painful sensation but does not cause tissue damage. The reliability of this device has been previously established (Hoeger Bement et al., 2008).

Data Analysis

The MVC force was quantified as the average value over a 0.5-s interval that was centered about the peak of the MVC. The maximal EMG for finger flexors and finger extensors was determined as the root mean squared (RMS) value over a 0.5-s interval about the same interval of the MVC torque measurement. The maximal EMG value measured during the handgrip MVC was used to normalize the RMS EMG values recorded during the fatiguing contraction for both the finger flexor and finger extensor muscles. Force fluctuations were quantified by normalizing the standard deviation of the force to the mean of the force [e.g. (SD of force/mean of

force)*100, (coefficient of variation (CV) of force)]. The RMS EMG of the finger extensor and flexor muscles and force fluctuations were measured during the fatiguing contraction at the following time intervals: the first and last 20 s of task duration and 10 s either side of 25%, 50%, and 75% of time to failure.

Heart rate and MAP were recorded at rest for one minute and during the fatiguing contraction and reported at start and end of task and at 25%, 50%, and 75% of time to failure. MAP was calculated for each time point with the following equation: MAP = DBP +1/3(SBP – DBP).

Statistical Analysis

Data are reported as means ± SD within the text and displayed as means ± SEM in the figures. Whenever two groups were compared, the Shapiro-Wilk test was performed to determine if data was distributed normally. When the data was distributed normally, independent t-tests were used to compare differences between groups. The Levene's test for equality of variance was used to test for homogeneity of variance between groups. When the normality of distribution within the data was not assumed, the Mann Whitney non-parametric test was used to observe differences between groups. Differences in control subjects and veterans with PTSD were compared for 1) various physical characteristics including, age, BMI and STAI (trait and state) levels, PCL-C and depression (BDI) scores, 2) time to failure, MVC force and percent reduction in MVC force, 3) baseline levels of heart rate and blood pressure and 4) differences in pain measures (present pain inventory, pain rating inventory, pain thresholds and average pain ratings during the 2 min task).

Two-way ANOVAs with repeated measures over time and group (PTSD vs. controls) as a between-subject factor were used to compare the various dependent variables. Repeated measure factors were performed separately for baseline to after fatigue (fatigue effect) and after fatigue throughout recovery (recovery effect), and during the fatiguing contraction (time effect, (0, 25, 50, 75, and 100% of time to failure)). Specifically, the statistical designs were as follows for the dependent variables: (1) time × group for force fluctuations, RMS EMG, MAP and heart rate during the fatiguing contraction, pain intensity during the 2-min pain test and (2) fatigue × group or recovery × group for comparison of MVC and levels of anxiety (VAS) before and after fatigue.

For the second aim, separate ANOVAs with repeated measures for time and session with the group (control vs. PTSD) as a between-subject factor, were used to compare various dependent variables. Separate ANOVAs were performed to compare control vs. the stressor session and control vs. mental-attentiveness session because not all subjects completed the mental-attentiveness session. Specifically, the statistical designs were as follows for the dependent variables: (1) session × group for time to task failure and percent reduction in MVC; (2) session × time × group for heart rate, MAP, force fluctuations, RMS EMG and RPE during the fatiguing contraction, (3) session × recovery × group for comparison of MVC. The strength of an association is reported as the squared Pearson product-moment correlation coefficient (r^2). A significance level of $P < 0.05$ was used to identify statistical significance.

A separate analysis was performed to determine if medications altered time to failure or reduction in strength. Similar procedures described previously were used to identify normality in data and then either the independent t-test or Mann Whitney U test

was conducted to determine differences between veterans taking medications (pain medications and anti-depressants) and veterans not taking medications.

An ANOVA with repeated measures for session (control vs. stressor) and group (medications vs. no medications) as a between factor were used to determine differences in time to failure, initial maximal strength (MVCs), reductions in strength (%), and force fluctuations (CV %). A separate ANOVA was performed for those taking pain medications and anti-depressant medications. Specifically, the statistical design consisted of session × group for time to task failure, percent reduction in MVC and maximal strength (MVC) and session × group and session × time × group for force fluctuations throughout the fatiguing contraction. Analysis was performed with SPSS (Version 19).

RESULTS

Subject characteristics. Veterans with PTSD were older by 8 years, and had a greater BMI, levels of trait anxiety, symptoms of PTSD and depression ($P < 0.05$, see Table 5.1). There was no difference in physical activity levels ($P > 0.05$) All veterans tested negative for the drug screen except two whom tested positive for opioids. Both of these individuals were on high levels of prescribed benzodiazepines (anxiolytic) which most likely contributed to a positive test. A medical doctor was consulted for advice regarding the positive tests. Twenty-four percent of the veterans were medication-free and 60 % were taking prescribed anti-depressant/anxiolytic medication (10 veterans taking selective serotonin reuptake inhibitors (SSRI), 4 of those veterans were also taking an anxiolytic medication. One veteran was taking a selective norepinephrine reuptake inhibitor (SNRI)). Fifty-five percent (11 veterans) were taking a prescribed pain medication.

Variable	PTSD	Control	P value (group effect)
Age (years)	36 ± 9	28 ± 9	$P = 0.005$
Body Mass Index (kg/m$^{2)}$	30.1 ± 4.5	23.7 ± 1.6	$P = 0.02$
PA (METS)	36.7 ± 42.3	59.1 ± 55.4	$P = 0.26$
Trait Anxiety (STAI)	57.2 ± 12.0	31.4 ± 5.1	$P < 0.001$
PCL-C	61.7 ± 13.3	24.6 ± 13.1	$P < 0.001$
BDI	31.7 ± 14.2	5.6 ± 8.9	$P < 0.001$

Table 5.1. Subject Characteristics for Veterans with PTSD and Control Subjects. The P value for each variable is indicated in the last column of the table. PA, Physical Activity Questionnaire; MET, Metabolic Equivalents·heart rate/week; PCL-C, Posttraumatic Stress Disorder Checklist-Civilian; BDI, Beck Depression Inventory

Aim 1: PTSD vs. Controls

Time to task failure and maximal strength. Time to task failure was briefer for veterans with PTSD compared with controls (27% difference between groups), ($P = 0.03$, Figure 5.2A). Time to failure was not associated with physical activity levels ($r = -0.14$, $P = 0.47$), BMI ($r = -0.31$, $P = 0.10$) nor the age of the individual ($r = 0.21$, $P = 0.13$). Maximal handgrip strength was similar across groups at baseline and throughout recovery ($P > 0.05$, Figure 5.2B). Reduction in strength was similar between groups (48 ± 14% for controls and 52 ± 14 % for veterans with PTSD, $P = 0.35$). Maximal strength was significantly lower than baseline values at 10 minutes recovery (recovery effect, $P < 0.001$) for both the veterans with PTSD (17 ± 9 %) and control group (15 ± 11 %) (time × group during recovery, $P = 0.73$) with no differences between groups (group effect, $P = 0.21$). Time to failure and strength was not different for veterans taking prescribed medications compared with those who were not taking medications (See Table 5.2).

Force fluctuations. Force fluctuations increased during the fatiguing contraction (time effect, $P < 0.001$) with no group effect ($P = 0.28$). However, the veterans with PTSD had a greater rate of increase in CV compared with the control group (0.82 ± 0.4 and 0.48 ± 0.3 $CV \cdot min^{-1}$, respectively, $P = 0.005$, Figure 5.2C). Force fluctuations tended to be greater at task failure for the veterans with PTSD compared with the control subjects ($P = 0.06$). The rate of increase in fluctuations was correlated with symptoms of PTSD (PCLC) (n=31, 10 controls, $r = 0.44$, $r^2=0.19$, $P = 0.02$).

Force fluctuations did not increase differently for veterans taking antidepressants compared with veterans not taking antidepressant medications (time × group, $P = 0.15$). There was no overall group effect ($P = 0.77$). Force fluctuations did increase more throughout the fatiguing contraction for veterans taking pain medications compared with veterans who were not taking pain medications (time × group, $P = 0.05$).

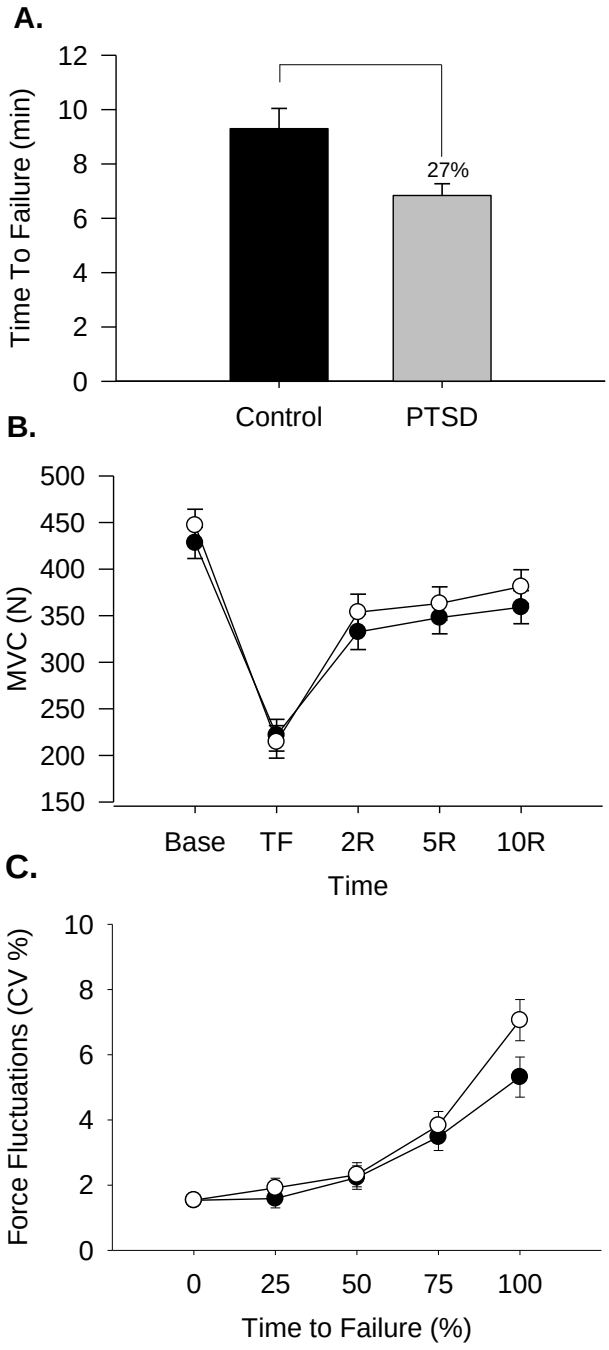

Figure 5.2. Time to Failure *(A)*, Maximal Voluntary Contractions (MVC) *(B)* and Force Fluctuations (CV %) *(C)* for Veterans with PTSD and Civilian Controls. Time to task failure for veterans with PTSD and control subjects for the 20% fatiguing contraction with the handgrip muscles. Veterans fatigued more quickly than control subjects ($P < 0.01$) ***(A)***. MVC force of the handgrip muscles for veterans with PTSD (open symbols) and control subjects (closed symbols) are shown at baseline (Base), at task failure (TF), and 2, 5 and 10 min throughout recovery (2R, 5R, 10R). Reduction in strength was similar for both groups ($P > 0.01$) ***(B)***. Force fluctuations for veterans with PTSD and control subjects throughout the fatiguing contraction. The rate of increase in force fluctuations was greater for veterans with PTSD ($P < 0.01$) ***(C)***. The values are presented as mean ± SE at 25% increments of the time to task failure.

EMG activity. Finger flexor and extensor EMG increased throughout the fatiguing contraction for both groups (time effect, $P < 0.001$) with no interactions ($P > 0.05$) and no group effects ($P > 0.05$) (Figures 5.3A and 5.3B).

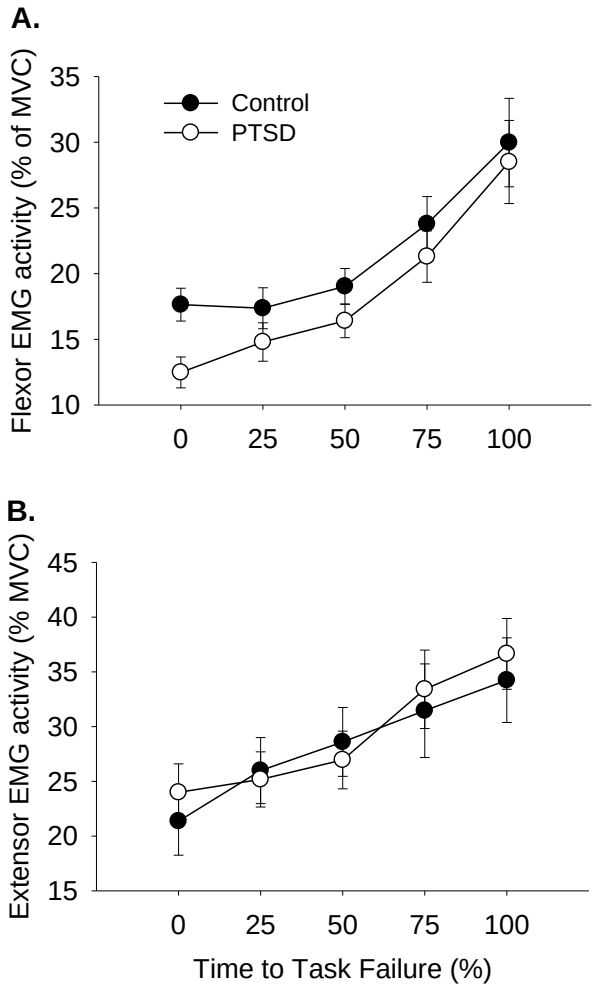

Figure 5.3. Root Mean Squared (RMS) EMG Activity for the Finger Flexors *(A)* and Finger Extensors *(B)* during the Fatiguing Contraction. RMS EMG of finger flexors and finger extensors normalized to the MVC values (% MVC) during the fatiguing contraction for veterans with PTSD (open circles) and controls (closed circles). Shown is the mean (± SEM) of 10 s intervals in 25% increments of the time to task failure.

Cardiovascular response during the fatiguing contraction. Heart rates were greater at baseline for veterans with PTSD compared with control subjects ($P = 0.002$, Figure 5.4A). Heart rate increased throughout the fatiguing contraction from baseline to task failure (time effect, $P < 0.001$) with a trend for heart rates to increase more for veterans ($P = 0.06$). Baseline heart rates correlated with symptoms of PTSD, quantified by the PCL-C (n = 31, 10 controls, $r = 0.48$, $r^2 = 0.23$, $P = 0.006$), suggesting that subjects with greater symptoms of PTSD have elevated levels of basal heart rates.

MAP was greater at baseline for veterans with PTSD compared with control subjects ($P = 0.04$, Figure 5.4B). MAP increased throughout the fatiguing contraction

(time effect, $P < 0.001$) similarly for both groups from baseline to task failure (time × group, $P = 0.93$). MAP was overall greater for those with PTSD (group effect, $P = 0.05$).

To understand the cardiac workload during the fatiguing contraction, rate pressure product was quantified (heart rate x MAP) (Wasmund et al., 2002; Yoon et al., 2009). The rate pressure product increased throughout the fatiguing contraction (time effect, $P < 0.001$, Figure 5.4C) but was greater for the PTSD group compared with the control subjects (group effect, $P = 0.05$).

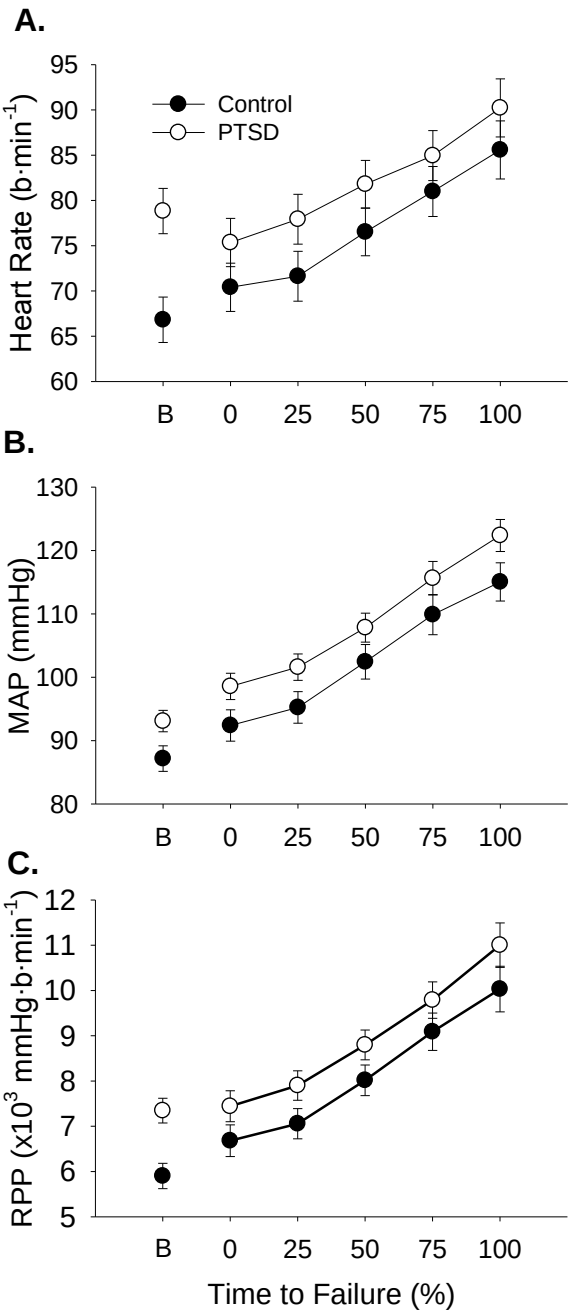

Figure 5.4. Heart Rate *(A)*, Mean Arterial Pressure (MAP;(*B*)), and Rate Pressure Product (RPP;(*C)*) for Veterans with PTSD and Civilian Controls during the Fatiguing Contraction. Heart rate, MAP and RPP were greater at baseline (B) for veterans with PTSD (open symbols) compared with control subjects (closed symbols) ($P < 0.05$). MAP and the RPP were greater throughout the fatiguing contraction for the veterans with PTSD compared with control subjects ($P < 0.05$). The values are presented as mean ± SE at 25% increments of the time to task failure.

Rating of perceived exertion. RPE increased throughout the fatiguing contraction (time effect, $P < 0.001$) similarly for both the control subjects and veterans with PTSD (time × group, $P = 0.50$) with no group effect ($P = 0.37$). Because time to failure was briefer for veterans with PTSD, the rate of increase was significantly greater for this

group (0.82 ± 0.13 min^{-1} for controls and 1.1± 0.14 min^{-1} for veterans with PTSD, $P < 0.001$).

Cognitive assessments of anxiety. State anxiety (STAI) was elevated for the veterans with PTSD at baseline and prior to the fatiguing contraction compared with the control subjects ($P < 0.001$). Levels of anxiety assessed by the VAS increased for both groups after the fatiguing contraction (fatigue effect, $P < 0.001$) with no interactions ($P > 0.05$). Levels of anxiety, however, were overall greater throughout the control session for the veterans with PTSD (group effect, $P = 0.04$). Trait (general) levels of anxiety correlated with symptoms of PTSD ($r = 0.92$, $r^2 = 0.85$, $P < 0.001$). The subjects who had greater symptoms of PTSD had greater general anxiety levels.

Aim 2: Influence of Acute Stressor on Fatigability

Time to failure. Time to failure was similar for the control and stressor session (7.9 ± 3 min vs. 8.4 ± 3 min, respectively, session effect, $P = 0.16$) for both groups (session × group effect, $P = 0.73$, Figure 5.5A). Veterans with PTSD had a reduced time to failure for both sessions compared with the control subjects (27% for control session and 21% for stressor session, group effect, $P = 0.05$). The mental-attentiveness task did not influence time to failure (session effect, $P = 0.34$) for either group (session × group effect, $P = 0.49$). Antidepressant and pain medications did not influence time to failure, maximal strength or the reduction in strength for the stressor session (See Table 5.2, $P > 0.05$)

Maximal strength. Maximal strength was similar between sessions (session effect, $P = 0.30$) for control subjects and veterans with PTSD (session × group effect, $P = 0.45$). Reduction in strength however, was greater for the control session compared with the

stressor session (session effect, $P = 0.02$, Figure 5.5B) for both control subjects and veterans with PTSD (session × group, $P = 0.31$) with no group effect ($P = 0.75$). MVC force was significantly lower than baseline values at 10 minutes recovery (time effect, $P < 0.001$) for both the control and stressor session (session × recovery, $P = 0.42$) with no differences between groups (group effect, $P = 0.21$). Neither maximal strength nor the reduction in strength was different for the control and mental-attentiveness sessions ($P > 0.05$).

Force fluctuations. Force fluctuations increased for both control and stressor sessions (time effect, $P < 0.005$). Fluctuations increased at a greater rate for the veterans with PTSD compared with control subjects (group effect, $P = 0.01$, Figure 5.5C).

Force fluctuations did not increase differently for those taking antidepressants compared with those who did not (time × group, $P = 0.32$) for either session (session × group effect, $P = 0.74$) with no session effect ($P = 0.37$) or group effect ($P = 0.82$). Force fluctuations did increase more throughout the fatiguing contraction for veterans taking pain medications for both sessions compared with veterans who were not taking pain medications (time × group, $P < 0.001$).

RMS EMG activity. Finger flexor and finger extensor EMG increased throughout the fatiguing contraction (time effect, $P < 0.001$, Figures 5.6A and 5.6B, control and stressor sessions only) for all three sessions ($P > 0.05$) with no effect of the stressor or mental-attentiveness task ($P > 0.05$) and no differences between the veterans with PTSD and the control subjects ($P > 0.05$).

Variable	Anti-depressant medication	No anti-depressant medication	Pain medication	No pain medication
Time to failure (min) (control)	7.3 ± 2.0	7.3 ± 3.4 *P* = 0.99	7.3 ± 2.0	7.4 ± 3.3 *P* = 0.90
Time to failure (min) (stressor)	7.2 ± 2.5	8.2 ± 3.0 *P* = 0.65	7.5 ± 2.3	7.8 ± 3.3 *P* = 0.84
MVC (N) (control)	441.1 ± 65.6	461.9 ± 94.8 *P* = 0.60	432.2 ± 68.2	470.5 ± 85.6 *P* = 0.30
MVC (N) (stressor)	444.8 ± 56.4	450.4 ± 83.4 *P* = 0.86	428.1 ± 55.5	470.2 ± 74.3 *P* = 0.18
Reduction in strength (%) (control)	51.8 ± 15.4	54.0 ± 13.3 *P* = 0.74	52.7 ± 14.7	52.6 ± 14.6 *P* = 0.99
Reduction in strength (%) (stressor)	41.4 ± 14.3	40.0 ± 20.5 *P* = 0.31	44.7 ± 12.9	45.8 ± 21.1 *P* = 0.87

Table 5.2. Motor Performance and Medications for Veterans. Time to failure, maximal voluntary contraction (MVC) strength and reduction in strength for the control and stressor session in veterans that take prescribed anti-depressant or pain medications compared with those who were not taking medications. P value is located in the second column for each variable and indicates the group effect.

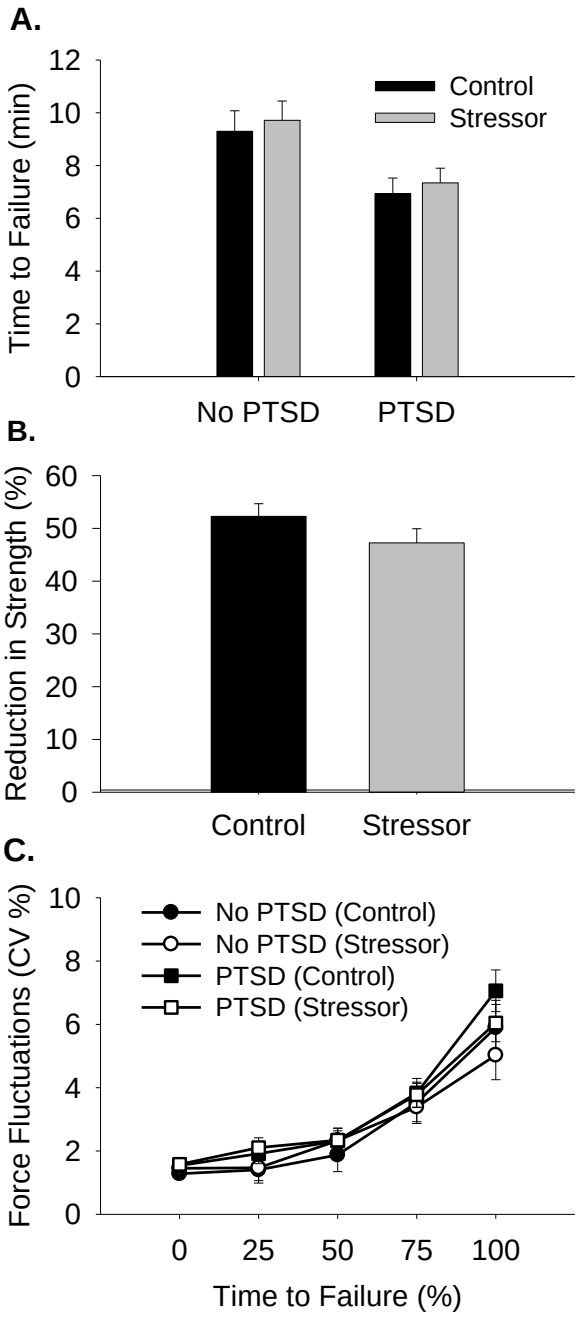

Figure 5.5. Time to Failure (*A*), Reduction in Strength (*B*) and Force Fluctuations (*C*) for Control and Stressor Sessions. Time to failure was similar for the control and stressor sessions ($P > 0.05$) but overall briefer for the veterans with PTSD ($P < 0.05$) ***(A).*** Reductions in strength for the control and stressor session were combined for the two groups. Reductions in strength were less for the stressor session compared with the control session for both veterans with PTSD and control subjects ($P < 0.05$) ***(B)***. Force fluctuations for veterans with PTSD (square symbols) and control subjects (circle symbols) throughout the fatiguing contraction for the control session (closed symbols) and the stressor session (open symbols). Force fluctuations were greater for veterans with PTSD ($P < 0.01$) ***(C)***. The values are presented as mean ± SE at 25% increments of the time to task failure.

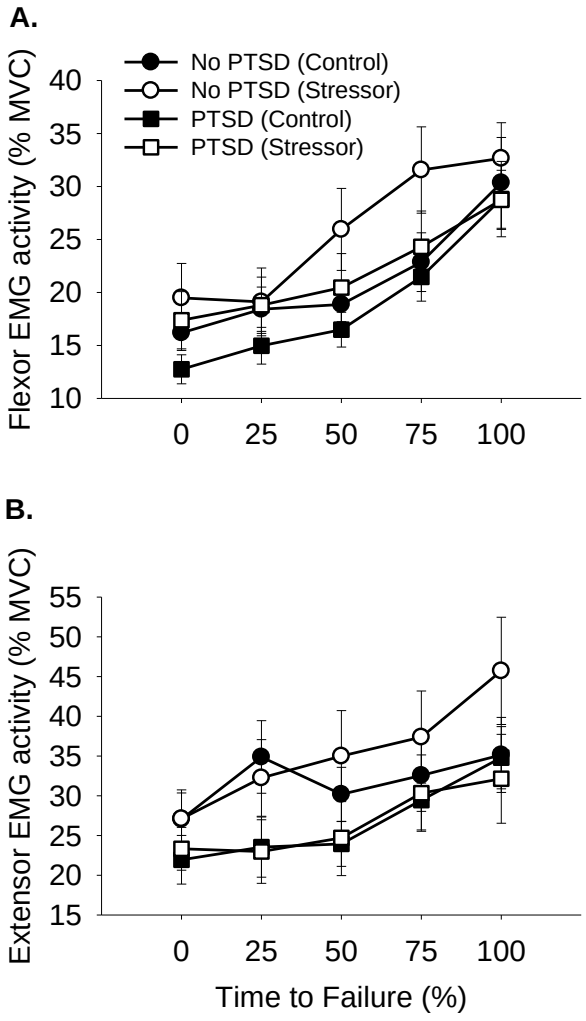

Figure 5.6. Root Mean Squared (RMS) EMG activity for the Finger Flexors *(A)* and Finger Extensors *(B)* during the Fatiguing Contraction. RMS EMG of finger flexors ***(A)*** and finger extensors ***(B)*** normalized to the MVC values (% MVC) during the fatiguing contraction. Shown is the mean (± SEM) of 10 s intervals in 25% increments of the time to task failure.

Cardiovascular response during the fatiguing contraction. Heart rates were overall greater for the stressor session compared with the control session (session effect, $P = 0.04$). Heart rate increased at a greater rate for the stressor session compared with the control session (session × time, $P = 0.003$, Figure 5.7A) for both the control subjects and the veterans with PTSD throughout the fatiguing contraction (session × group, $P = 0.13$).

Mean arterial pressure increased throughout the fatiguing contraction (time effect, $P < 0.001$) similarly for both the control subjects and the veterans with PTSD (session × group, $P = 0.91$, Figure 5.7B) with a trend for MAP to be greater during the stressor session (session × time, $P = 0.06$) with no overall effect of session ($P = 0.11$) or group (P

= 0.28). The rate pressure product increased more for the stressor session than the control session for both groups (session × time, $P = 0.001$, Figure 5.7C) with no group effect ($P = 0.21$).

Rating of perceived exertion. RPE increased throughout the fatiguing contraction for all three sessions (time effect, $P < 0.001$). The rate of increase was less during the stressor session (session effect, $P = 0.004$) but overall greater for the veterans with PTSD for both sessions (group effect, $P < 0.001$).

Cognitive assessments of arousal. For both sessions, state anxiety was greater for veterans with PTSD (group effect, $P < 0.001$). Anxiety increased after exposure to the cognitive stressor (session × time, $P < 0.001$) similarly for the control subjects and veterans with PTSD (session × time × group, $P = 0.72$). Levels increased from 45.4 ± 11.1 to 58.0 ± 9.4 for veterans with PTSD and 30.4 ± 7.6 to 40.6 ± 9.2 for the control subjects.

Baseline anxiety (VAS) was also higher for veterans with PTSD compared with control subjects (2.3 ± 2.1 and 1.0 ± 1.3, respectively). Anxiety (VAS) increased after the cognitive stressor compared with quiet sitting (50 % increase for control subjects and 34 % increase for the veterans with PTSD, session × time, $P < 0.001$) and after the fatiguing contraction (compared to baseline measures) while simultaneously performing the stressor task (56 % increase for control subjects and 45% increase for the veterans with PTSD, session × fatigue, $P = 0.01$). Anxiety (STAI or the VAS) did not increase for either group the for the mental-attentiveness task ($P > 0.05$).

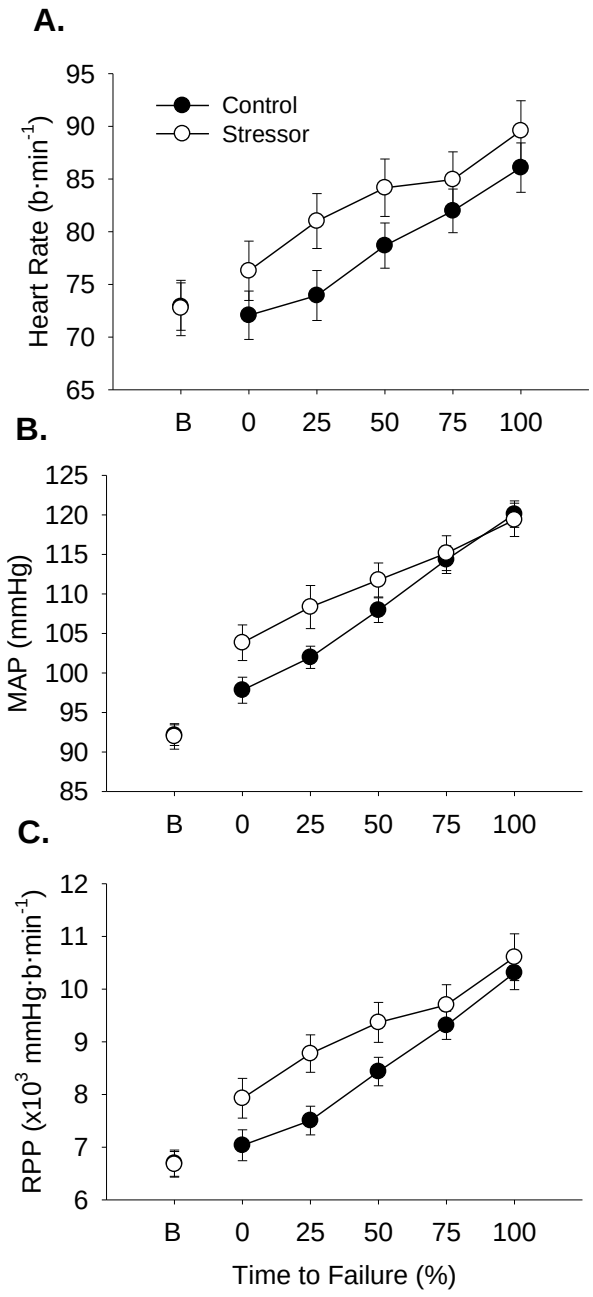

Figure 5.7. The Heart Rate *(A)*, Mean Arterial Pressure (MAP;(*B*)), and Rate Pressure Product (RPP;(*C)) for the Control (closed symbols) and Stressor Sessions (open symbols) for the Combined Groups. Heart rate and RPP were greater throughout the stressor session for both groups ($P < 0.05$). There was a trend for a greater MAP throughout the stressor session ($P = 0.06$) compared with the control session. The values are presented as mean ± SE at 25% increments of the time to task failure. The disconnected symbol indicates baseline (B) values for each variable.

Pain Assessments

2-minute pain test. Pain thresholds quantified by the pain/pressure device were lower for the veterans with PTSD compared with control subjects ($P = 0.02$, Figure 5.8A). Pain ratings (rated every 20 seconds) did not increase throughout the 2-min test (time effect, $P = 0.22$) for the control and PTSD subjects (time × group, $P = 0.08$) and

average pain ratings were similar for the two groups ($P = 0.13$). Pain thresholds correlated with scores on the PCLC ($r = -0.60$, $r^2 = 0.36$, $P = 0.02$) but not BDI scores ($r = -0.48$, $r^2 = 0.23$, $P = 0.08$), indicating that those who had more severe symptoms of PTSD, but not depression, felt pain more quickly.

McGill pain questionnaire. Pain rating inventory and present pain intensity, quantified by the McGill pain questionnaire were greater in veterans with PTSD compared with control subjects ($P < 0.05$, Figures 5.9A and 5.9B) The present pain intensity score was associated with the PCLC ($r = 0.61$, $r^2 = 0.37$, $P = 0.02$) and BDI ($r = 0.61$, $r^2 = 0.37$, $P = 0.02$). Time to failure was negatively correlated with the pain rating inventory quantified by the McGill pain questionnaire indicating that those that had higher pain ratings had a reduced time to failure ($r = -0.66$, $r^2 = 0.44$, $P = 0.01$, Figure 5.8B). Neither the present pain intensity scale nor the pain rating inventory was associated with pain thresholds ($P > 0.05$).

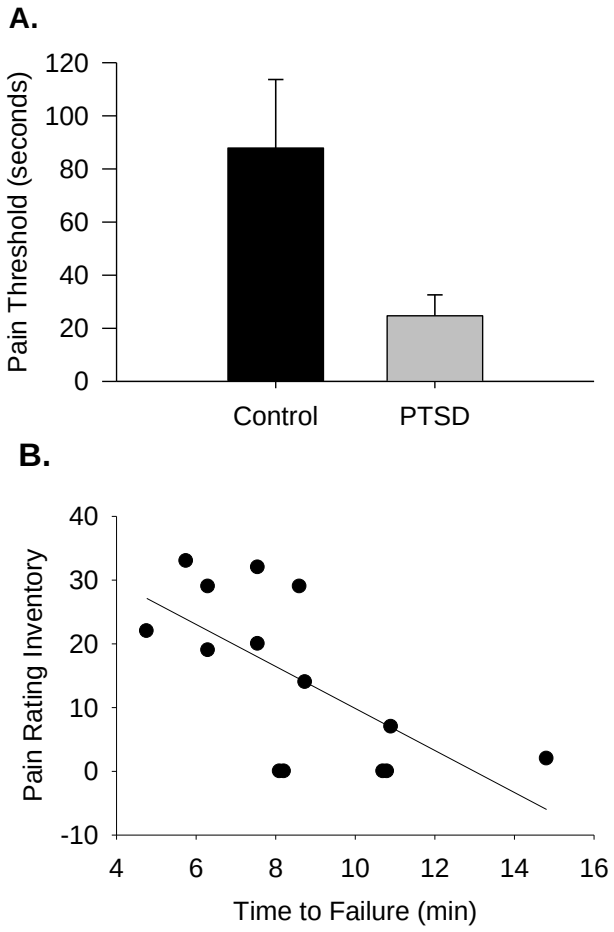

Figure 5.8. Pain Thresholds *(A)* and Association between Time to Task failure and McGill Pain Rating Inventory Scale (*B*). Pain thresholds were assessed by a pain/pressure device for 2 minutes. Pain thresholds were less for veterans with PTSD ($P < 0.01$) ***(A)***. There was an association between time to failure and pain ratings quantified by the McGill pain questionnaire ($r = -0.66$, $r^2 = 0.44$, $P = 0.01$) ***(B)***.

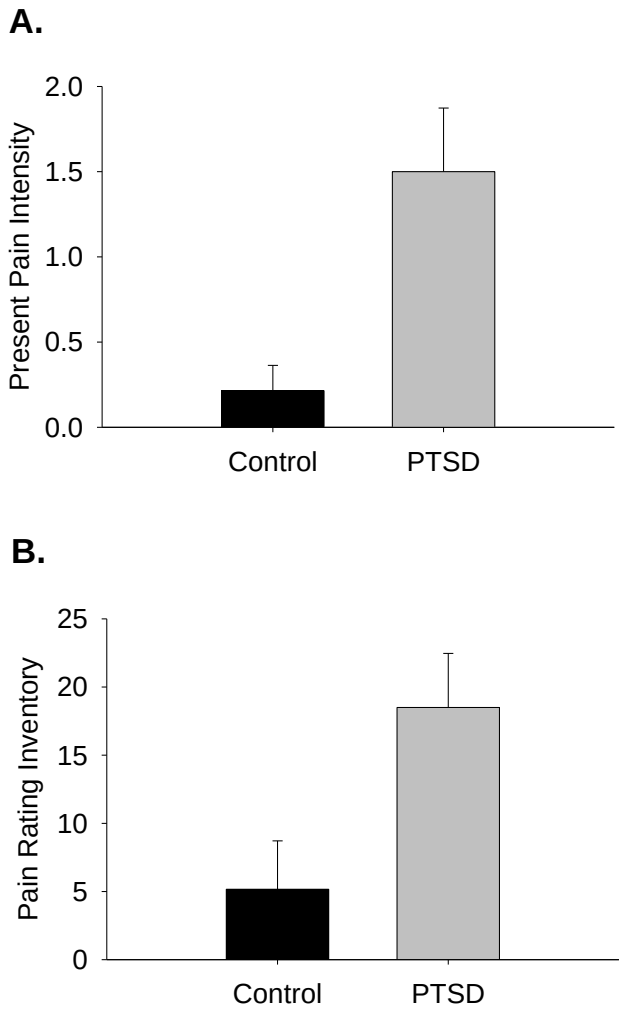

Figure 5.9. Present Pain Intensity (A) and Pain Rating Inventory for Veterans with PTSD and Control Subjects. The present pain intensity and pain rating inventory were assessed by the McGill pain questionnaire. Veterans with PTSD had greater pain intensity scores and ratings of symptoms of pain compared with the control subjects $P < 0.05$).

DISCUSSION

This study investigated the fatigability and steadiness of the hand muscles in veterans with PTSD in the presence and absence of an acute stressor. The novel findings from this study were that 1) fatigability was greater and force fluctuations increased at a greater rate for veterans with PTSD compared with control subjects for a low-intensity contraction, 2) the acute stressor did not alter time to failure or force fluctuations for veterans with PTSD or control subjects, 3) veterans with PTSD had lower pain thresholds compared with control subjects in response to a pain/pressure device and reported greater

pain ratings and intensity of pain at rest (McGill)(Melzack, 1975) and 4) those with greater reports of pain had a briefer time to task failure.

As part of the inclusion criteria for the PTSD group, a medical diagnosis of PTSD was required by a physician. To quantify the veterans' current self-report of PTSD symptoms, veterans filled out the PCL-C during their initial visit. As expected, symptoms of PTSD were significantly higher in veterans with PTSD compared with the control group. Because depression is often comorbid with PTSD (Koenen et al., 2008), symptoms of depression were quantified by the BDI. This cohort of PTSD subjects on average had moderate levels of depression (index of ~ 30). Eleven veterans were taking prescribed anti-depressants medications (4 out of the 11 were also taking anxiolytics), all but one were taking selective serotonin reuptake inhibitors (SSRIs) and the veteran not taking the SSRI was taking a selective norepinephrine reuptake inhibitor (SNRI). Pain is also comorbid with PTSD (McGeary et al., 2011). Eleven out of the 20 veterans were taking pain medications in this study. Veterans taking pain medications had similar MVCs, reductions in strength and time to failure, but did have greater increases in force fluctuations during the fatiguing contraction for both the control and stressor session compared with those not taking pain medications (discussed below).

SSRI's reduce the reuptake of serotonin in the brain, enhancing brain serotonergic activity and improving mood and cognition (Krystal & Neumeister, 2009). Serotonin is also important for motor function (Loubinoux et al., 2002) and has been implicated in improving motor performance in those post-stroke (Pariente et al., 2001; Loubinoux et al., 2002; Chollet et al., 2011). Therefore, it might be expected that the veterans taking SSRIs would have enhanced performance (greater maximal strength, less reductions in

strength, longer time to failure and less motor output variability). We however, did not find a difference in performance between those taking the medications and those who were not. This is consistent with a study that investigated the effect of both acute and chronic SSRIs on maximal strength and high-intensity exercise (cycling test) in individuals without motor dysfunction (Parise et al., 2001). The increased performance with serotonin use therefore might be specific to the motor impairment.

PTSD can result from exposure to one or more acute traumatic stressors. Veterans with a diagnosis of PTSD in this study demonstrated elevated basal levels of anxiety (VAS and STAI-state and trait), heart rate and mean arterial pressure. These results are consistent with previous studies (Bedi & Arora, 2007). Additionally, individuals with greater symptoms of PTSD had greater levels of baseline heart rates. Importantly, a multi-site study demonstrated that heart rate was a predictor for having and/or developing PTSD (Bryant et al., 2008). Elevated heart rates and blood pressures are known to be indices of sympathetic activation (Ettinger et al., 1996) and are related to greater plasma levels of serotonin, norepinephrine and epinephrine (neuromodulators) (Southwick et al., 1999; Bedi & Arora, 2007). Though these neuromodulators were not measured in this study, it is likely that they were higher at baseline in the veterans with PTSD. Indices of sympathetic activation and the rate pressure product were also greater throughout the fatiguing contraction (marginal significance for heart rate). The elevated rate pressure product suggests a greater energy demand and oxygen consumption of the heart (Wasmund et al., 2002). Collectively, these results suggest that at baseline and throughout the fatiguing contraction for the control session, veterans with PTSD may have had greater levels of sympathetic activation.

PTSD is Associated with Greater Fatigue and Reduced Steadiness

To our knowledge this is the first study demonstrating decrements in motor performance for veterans with PTSD for a fatiguing motor task. Despite the fact that the groups were fatigued to the same magnitude (similar reduction in strength) veterans with PTSD were unable to sustain the contraction for as long as the control subjects. When healthy young adults are exposed to an acute stressor, the time to failure difference was associated with the maximal strength of the individual (Yoon et al., 2009) and were paralleled by greater indices of sympathetic activation. Sympathetic activity in those with PTSD is altered (Southwick et al., 1999) and may impact motor function. Greater sympathetic activity is associated with changes in the contractile force of the muscle (Bowman, 1980; Roatta et al., 2008), increased vasoconstriction by norepinephrine binding to α adrenergic receptors, reduced blood perfusion to the muscle (Thomas & Segal, 2004) and altered proprioceptive feedback to the motoneuron in the spinal cord (Roatta et al., 2002; Hellstrom et al., 2005). Based on previous findings in young healthy men and women (Yoon et al., 2009), it is possible that the greater muscle fatigability in veterans with PTSD is caused by greater vasoconstriction, reducing the amount of blood perfusion and therefore oxygen supply to the exercising muscle.

Alternatively, other mechanisms need to be considered. PTSD is a centrally mediated disorder and has been associated with changes in brain structure, size and function. Changes are observed in the hippocampus (Felmingham et al., 2009), amygdala (Liberzon & Phan, 2003) and prefrontal cortex (Karl et al., 2006; Eckart et al., 2011) and these changes are known to be associated with a decrease in the norepinephrine and serotonin transmission in those with PTSD (Southwick et al., 1999). Not only are these

centers involved in the stress response and altered by adaptations from traumatic stress (McEwen, 2007), but they are also involved in several other functions including motivation, executive function, fear response, learning and working memory, all of which are altered in people with PTSD (Krystal & Neumeister, 2009).

The motor cortex, though outside of the circuitry that is usually involved with the manifestations of PTSD, is functionally connected to the circuitry affected by PTSD. The motor cortex exhibits altered excitability and inhibition in resting conditions and during voluntary contractions of the first dorsal interosseous muscle in PTSD patients (Rossi et al., 2009). This was assessed with TMS, which is a particularly sensitive technique for disclosing early subclinical changes of the motor system (Rossi et al., 2009). The central nervous system necessitates increases in both excitatory and inhibitory activity during fatiguing contractions, and an imbalance of excitation in the corticomotor pathway in people with PTSD may lead to impairments in motor performance.

Motor performance in PTSD is not exacerbated by acute cognitive stress

Contrary to our expectations, neither the veterans with PTSD nor the control subjects demonstrated a decrement in time to failure when exposed to the acute cognitive stressor. Possible reasons for this are that 1) the data analyzed in this study involved men and/or 2) the greater fatigability when exposed to a cognitive stress may be dependent on the muscle group. A reduced time to failure when exposed to a stressor is shown for the elbow flexor muscles (Yoon et al., 2009), but the acute stress does not appear to have a similar effect in the handgrip muscles. Nonetheless, time to failure was reduced by 27% in the veterans with PTSD for the control session and by 21% in the stressor session when compared with the control subjects. This may indicate a stronger influence of the

chronic stress condition (overactive sympathetic drive and/or corticomotor hyperexcitability) (Southwick et al., 1999; Rossi et al., 2009) on motor performance for low-intensity fatiguing tasks.

A surprising finding in this study was that the reduction in strength was less for the stressor session compared with the control session for both groups, despite no differences in time to failure across sessions. Anxiety, heart rate and mean arterial pressure were greater during the fatiguing contraction in the stressor session compared with the control session. This indicates that the acute activation of sympathetic activity was greater for the stressor session and likely similar for the control and PTSD group. Evidence suggests that increases in sympathetic activation will potentiate (increase force) Type II fibers (Bowman, 1980) and increase the contractility of the muscle. Therefore, during a high-intensity contraction, where Type II fibers are activated, the stress-induced sympathetic activation may potentiate the force leading to greater muscle activation and stronger MVCs after the fatiguing contraction.

Pain, physical activity and PTSD

Pain perceptions and pain processing are known to be altered in those with PTSD (Geuze et al., 2007; Cho et al., 2011; McGeary et al., 2011). The novel findings from this study demonstrate that the greater fatigability for the control session for subjects with and without PTSD was associated with pain ratings on the McGill pain questionnaire. Furthermore, pain thresholds (pain/pressure device) were less for those with PTSD, suggesting hypersensitivity to pain in veterans with PTSD compared with control subjects. These findings are in contrast to studies that use a thermal stimulus in patients with PTSD (Defrin et al., 2008; Kraus et al., 2009). Pain thresholds were not dependent

on whether the individual (neither veteran's with PTSD or control subjects) had higher pain ratings or intensity of pain (indicated by the McGill questionnaire) and were also not associated with having depression. This suggests that the hypersensitivity to pain is more closely linked with symptoms of PTSD, but not depression and is not dependent on the chronic pain levels for this group of subjects.

Force fluctuations increased at a greater rate in veterans with PTSD. The rate of increase was associated with the severity of PTSD symptoms (PCL-C) but was greater for veterans that were taking pain medications. Because 60% of the veterans with PTSD were taking pain medications and still experiencing pain, it is difficult to determine which might be more relevant to the greater amplitude of force fluctuations. Currently, there is limited information in the literature regarding how pain medications may influence motor performance. A recent study indicated that chronic opioid use in rats resulted in decreased grip strength (Mitzelfelt et al., 2011), but there was no difference in strength between the veterans taking pain medications and those who were not taking pain medications in the current study.

There is some evidence, however, that pain may alter force fluctuations. Stimulation of nociceptors (pain receptors) can decrease the discharge rate of motor units during sustained contractions (Farina et al., 2005). Reducing the discharge rate of motor units can increase the variability of the discharge rate and consequently lead to greater amplitudes of force fluctuations (Taylor et al., 2003). Greater activation of nociceptors in veterans that experience more chronic or acute pain may alter the force fluctuations during the fatiguing motor task. Veterans with more chronic pain may have experienced

more acute pain during the fatiguing contraction that could have influenced modulation of force fluctuations.

Physical activity levels are shown to be reduced in patients with PTSD (de Assis et al., 2008); however, in this study, there were no differences in physical activity levels nor was physical activity associated with time to failure. BMI was higher in those with PTSD, but BMI was also not associated with briefer time to failure. Thus, veterans with PTSD were not more fatigable because they were less active or more overweight.

Limitations

This study has limitations that should be considered before generalizing the results to all veterans with PTSD. One limitation was that the control group was composed of civilian subjects. An ideal comparison group would be combat veterans without PTSD. Combat veterans without PTSD were actively recruited, four were familiarized, two did not finish the study because of work-related obligations. Two were diagnosed with PTSD after being familiarized into the study. Another limitation of the study is that the data is representative of only men. Female veterans were also actively recruited, and as a small number of women demonstrated interest, only one fit the criteria for the study and therefore her data was not included in the analysis.

A third limitation of the study was the comorbidity of depression in the veteran population. To determine if the greater reductions in steadiness and increased fatigability are purely from having PTSD and not depression, future studies could include a group that is diagnosed with only depression or PTSD without depression, although a population of PTSD without depression would be difficult to recruit.

Finally, we did not control for medications and smoking behavior in the men with PTSD. Because results indicated that pain and anti-depressant medications did not contribute to the greater fatigability in veterans with PTSD, recruitment of veterans that are free of medications might lead to a more clear interpretation of the results. Additionally, the effect of smoking on motor performance is not known and may be a factor in this study.

Conclusion

In summary, the results from this study demonstrate that fatigability is greater and steadiness is reduced in veterans with PTSD. Although the mechanisms are not understood, three possibilities for the greater fatigability are 1) dysfunctions in corticomotor excitability, 2) sympathetically-induced reduced blood perfusion to the exercising muscle or 3) greater chronic pain and more acute pain during the fatiguing contraction. Furthermore, fatigability was not greater when exposed stress compared with the control session, which may be due to differences in muscle groups or sample of subjects (men vs. women). Time to failure was less for veterans with PTSD in both the control and stressor sessions compared with the non-PTSD group. This may indicate that the influence of the chronic stress (long-term alterations in sympathetic activation) may have a stronger influence than the acute stress for fatiguing motor tasks in men.

This study is clinically important because it attempts to expose altered motor control in clinical populations. Patients with PTSD develop dysfunctions in the regulation of stress systems, and this study suggests that they may also demonstrate impairments within the neuromuscular system.

Chapter VI

General Discussion

This thesis investigated how acute stress in healthy men and women and PTSD in veteran males can alter muscle fatigability and motor output variability of low-intensity contractions in the upper limb muscles. Muscle fatigue is the foundation for rehabilitation and the interaction of fatigue and stress is important in understanding causes of musculoskeletal disorders in both healthy individuals and clinical populations. The findings from these studies provide new insight into neuromuscular adjustments for low-intensity motor fatiguing tasks, which are the basis of functional activities. Furthermore, the results from this thesis contribute to the current literature by demonstrating a distinct relationship between stress systems and the motor system. This chapter will discuss the novel findings of the dissertation thesis, significant contributions to the current literature and implications for future research.

Muscle fatigue characteristics and motor output variability differ depending on the specific requirements of the task, the environment in which the task is performed and the individual executing the task (Enoka & Duchateau, 2008). Consistent with the task specificity of fatigue, this thesis demonstrated that for low-intensity sustained contractions 1) men and women fatigue more quickly when exposed to an acute cognitive stressor (21 % difference in time to failure for women and 10 % difference in time to failure for men) and 2) male combat veterans with PTSD are more fatigable than strength-matched civilian men without PTSD in the presence (21%) and absence of an acute cognitive stressor (27%).

The Influence of Acute and Chronic Stress on Motor Performance

The acute stress response results in a sudden increase in sympathetic activation (Herd, 1991) and having PTSD is associated with greater chronic levels of sympathetic activation (Southwick et al., 1999). Some of the manifestations include transient increases in heart rate and MAP with acute stress in a healthy population (Chapters III and IV) and chronic basal elevations of heart rate and MAP in veterans with PTSD (Chapter V). Therefore, the greater sympathetic activity that occurs in both the acute and chronic stress condition may influence motor performance similarly. Thus, the acute stress-induced reductions in time to failure in healthy adults and the briefer time to failure shown in veterans with PTSD may be of similar mechanisms.

The results from Chapter V also demonstrate that neither the veterans with PTSD nor control subjects were more fatigable when exposed to an acute cognitive stressor. Consequently, this indicates that motor performance may either be more affected by acute stress in women (Yoon et al., 2009) or that the handgrip muscles are less sensitive than the elbow flexor muscles to the acute increases in sympathetic activation. Furthermore, the veterans with PTSD were more fatigable in both conditions suggesting that the influence of the chronic stress condition may be greater than the acute stress condition on motor performance in this clinical population.

What is currently understood regarding how sympathetic activation can influence motor performance has been shown in healthy human populations or animal models, but has not yet been demonstrated in clinical populations. The current evidence suggests that the acute increase in sympathetic activation will result in a release of epinephrine, norepinephrine and serotonin and can alter motor performance in the following ways.

Sympathetic activation can 1) reduce blood perfusion to the muscle by norepinephrine binding to α-adrenergic receptors on the arterioles and causing vasoconstriction of the blood vessels (Thomas & Segal, 2004), 2) increase blood perfusion to the muscle by β-adrenergic activation of the arterioles (Joyner & Dietz, 2003), 3) reduce afferent (muscle spindle) feedback to the α motoneuron (Roatta et al., 2002; Hellstrom et al., 2005), 4) alter contractility of the force of muscle fibers; weaken Type I and potentiate Type II fibers (Bowman, 1980; Roatta et al., 2008) and 5) alter the synaptic activity at the α motoneuron, altering the input-output gain and increasing excitability of the motoneuron (Heckman et al., 2003). The reduced time to failure that occurred with exposure to an acute stress in young healthy men and women (Chapter III) and the briefer time to failure that occurred in veterans with PTSD (Chapter V), could therefore be a result of the influence of sympathetic activity on motor control at these various sites within the neuromuscular system.

Transcranial Magnetic Stimulation (TMS) is a multi-faceted technique and was used in this dissertation to understand mechanisms of stress-induced muscle fatigue in healthy adults. Using single-pulse stimulation, both central and peripheral nervous system adjustments with fatigue and stress can be assessed including; supraspinal fatigue, corticomotor excitation and inhibition and contractile properties of the muscle (peak relaxation rates and estimated resting twitch). However, electrical stimulation has been used in previous studies to induce stress (Noteboom et al., 2001b; Christou et al., 2004). As a result, Chapter II confirmed that TMS, along with brachial plexus stimulation (information regarding neuromuscular propagation), can reliably quantify adjustments in

the neuromuscular system without causing alterations in motor performance (greater reductions in strength, reduced time to failure or greater motor output variability).

Because sympathetic activation can alter muscle fiber types differently and women demonstrate a greater proportion of Type I fibers (Simoneau & Bouchard, 1989) it was hypothesized that the peak relaxation rates (quantified by TMS) would decline (slow) more for the women than the men after exposure to a cognitive stressor. Additionally, because the stress response is also centrally-mediated and there are sex differences in the stress response (Carvalho-Netto et al., 2011), it was expected that supraspinal fatigue (quantified by TMS) would contribute to the greater fatigability when exposed to the cognitive stressor and that this may be different for men and women. In contrast to these expectations, the stress-induced muscle fatigability in healthy young men and women was not caused by any differences in supraspinal fatigue or activation of the peak relaxation rates. Importantly, results from Chapter III also demonstrated that corticomotor excitability and inhibition was also not altered after the fatiguing task when exposed to the acute stress.

The central nervous system necessitates increases in both excitatory and inhibitory activity during fatiguing contractions (Gandevia, 2001) and an imbalance may impair motor performance. Patients with PTSD demonstrate impaired corticomotor inhibition and exaggerated corticomotor excitation (by use of paired-pulse TMS) (Rossi et al., 2009). This is important because it distinguishes that the corticomotor system, although not involved with the acute stress response, is altered with chronicity of stress. Therefore, centrally-mediated mechanisms of increased fatigability for the veterans with PTSD cannot be ruled out and deserves further exploration in future studies.

Chapter III demonstrated that the reduced time to failure with exposure to an acute stressor was associated with the strength of the individual. Women (who are usually weaker than men) contract at a lower absolute force (for a relative contraction) and have greater blood perfusion to the exercising muscle during a fatiguing contraction than men (Hunter et al., 2006b). Although blood perfusion was not measured in this study, it is possible that women or weaker subjects may be more sensitive to stress-induced alterations in blood perfusion to the exercising muscle during stressful conditions compared with men or stronger subjects. Although veterans with PTSD were strength-matched and time to failure was not associated with strength, they usually demonstrate greater levels of plasma neuromodulators than healthy controls (Southwick et al., 1999) and therefore greater sympathetic activity. Thus, it is possible that the greater fatigability in veterans with PTSD may be due to a sympathetic-induced vasoconstriction to the exercising muscle causing reduced blood perfusion during the fatiguing motor task.

When studying clinical populations, other factors such as co-morbidities need to be considered. Pain is co-morbid with PTSD. Pain ratings on the McGill questionnaire were associated with time to failure for the veterans with PTSD and control subjects. The association suggested that the individuals that experienced greater pain ratings had a briefer time to failure. It is possible that those with chronic pain experienced more acute pain during the fatiguing contraction, and therefore pain may have contributed to a briefer time to task failure. Thus, the influence of pain on motor control in people with PTSD is not understood and the contributions of pain to a briefer time to failure warrant further investigation.

Summary

Results from Chapter III indicate that the reduced time to failure with exposure to acute stress was not due to supraspinal fatigue or alterations in the peak relaxation rates but is associated with the strength of the individual. It is hypothesized that stress-induced fatigability in the weaker subjects may be due to a greater vasoconstriction and less blood perfusion to the exercising muscle during the motor task. Because of the greater sympathetic drive in veterans with PTSD, it is hypothesized that the mechanism for the greater fatigability in the veterans with PTSD is also likely due to reduced blood perfusion during the fatiguing contraction. Future studies are necessary to examine and provide evidence for this proposed mechanism. Using techniques such as ultrasound, venous occlusion plethysmography and measurements of sympathetic activity at the peripheral nerve (MSNA) will lead to a better understanding of how increased sympathetic activity and alterations in blood perfusion may contribute to a reduced time to failure when exposed to an acute stressor in healthy young adults and a briefer time to failure in veterans with PTSD.

This integrative and unique approach in studying fatigability in a clinical population such as PTSD is advantageous because it compels scientists to consider how the stress and motor systems may interact and particularly how physiological adaptations in one system may influence another system. Furthermore, understanding the mechanisms of fatigability will generate rehabilitation strategies and development of optimal treatment intervention programs tailored to specific populations and/or motor impairments.

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